

THE NATIONAL EYE INSTITUTE

ANNUAL REPORT

Fiscal Year 1982

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STATEMENT OF THE INSTITUTE DIRECTOR

In FY 1982 the National Eye Institute's budget increased to \$127 million, enabling the NEI to support 1,075 extramural research project grants and 74 intramural projects. The number of grants was almost as many as the 1,100 funded in FY 1979, the most grants NEI ever supported in one year.

One of the most important management activities of the NEI during the past year was the development of the National Advisory Eye Council's third comprehensive program evaluation and national plan for FY 1983-1987. This multivolume report, which includes contributions from staff in every branch of the Institute, will be published early in 1983 and will provide the Council and NEI staff with guidance for the development of the nation's vision research effort over the next few years. The new plan is expected to be of great value to the NEI staff in formulating policy and in carrying out the day-to-day management of NEI programs. We also expect the new report to increase the Institute's capability to respond to changing national policies concerning Federal support of biomedical science. It is clear that program planning will be even more crucial in the years to come than it has been in the past as the Administration and the Congress reexamine the Federal role in supporting a wide range of domestic programs. In the meantime, the NEI will make every effort to maximize the return on its investment in vision research and to modify its policies and organization to meet changing needs.

An important breakthrough in the prevention of blindness was made during the past year when NEI-supported investigators participating in the Senile Macular Degeneration Study observed, after only 3 years of the planned 5-year clinical trial, a significant pattern in results. Sixty percent of the untreated eyes with the neovascular form of the disease lost most of their vision, while only 25 percent of the treated eyes had such an outcome. These results were announced at a well-attended news conference held on the NIH campus in May of this year and a full report of the Study findings was subsequently published in the Archives of Ophthalmology in June. Since that time additional efforts have been made to communicate the significance of the study's findings to health professionals and the population at risk to senile macular degeneration.

An important activity of the National Eye Institute over the past year has been the Second General Assembly of the International Agency for the Prevention of Blindness. In October of this year the NEI will act as host to approximately 300 people from more than 50 countries who will come to NIH to participate in the largest international gathering of experts on blindness prevention that has ever been convened.

A significant addition to the National Eye Institute staff has resulted from the appointment of Barbara Underwood, Ph.D., an international expert on nutrition, as Special Assistant to the Director for Nutrition Research. In her new position, Dr. Underwood will coordinate Institute programs in nutrition and will pursue her own research on the relationship of vitamin A and nutrition to blindness and severe visual impairment.

One of the major administrative accomplishments of FY 1982 was the opening of NEI facilities in the new NIH Ambulatory Care Research Facility, including the initial phase of moving the Laboratory of Sensorimotor Research from various temporary quarters to a consolidated laboratory. This move will not only help create a better scientific milieu for these investigators, it will produce a greater level of collaboration among scientists conducting laboratory studies of eye movements and clinicians investigating oculomotor disorders in humans.

The accomplishments of the NEI and those it supported over the past year, and examples of current and planned research and administrative endeavors, are highlighted in the following reports of NEI offices and branches.



Carl Kupfer, M.D.

EXTRAMURAL AND COLLABORATIVE PROGRAMS



ANNUAL REPORT
NATIONAL EYE INSTITUTE
October 1, 1981 - September 30, 1982

REPORT OF THE ASSOCIATE DIRECTOR FOR EXTRAMURAL AND COLLABORATIVE PROGRAMS
Ronald G. Geller, Ph.D.

In keeping with the Institute's first priority, support for investigator-initiated individual research projects (R01 and R23), 955 awards were made covering a wealth of scientifically exciting ideas relevant to the prevention, treatment, and cure of diseases and disabilities of the visual system. This represents about 90 percent of our extramural budget. The following sections highlight some of the issues and accomplishments in the NEI Extramural and Collaborative Programs during FY 1982.

For FY 1982, the National Eye Institute received an appropriation of \$127,374,000--an increase of \$9,391,000 over the previous year's appropriation. Of the \$127,374,000, a total of \$109,606,000 was allocated to Extramural and Collaborative Program activities in the following categories:

Research Grants	\$ 99,126,000
Research Training Awards	3,446,000
Research Contracts	5,034,000
Construction Grants	2,000,000
Total	<u>\$109,606,000</u>

These funds were distributed among the Institute's five research programs as follows:

Research, Training, & Contract Dollars (in thousands)	
Retinal and Choroidal Diseases	\$ 45,625
Corneal Diseases	18,146
Cataract	10,542
Glaucoma	10,610
Strabismus, Amblyopia, and Visual Processing	22,683
Total	<u>\$107,606</u>

The Institute was able to fund 50 percent of all approved applications, essentially the same as in FY 1981. The data are given below:

Grant Application Rate¹

	<u>Received & Reviewed</u>	<u>Recommended For Approval</u>	<u>Approved & Funded</u>	<u>% Funded of All Approved Applications</u>
FY 1978	681	562	343	61
FY 1979	579	495	308	62
FY 1980	516	432	225	52
FY 1981	636	606	309	51
FY 1982	629	553	275	50

¹ R01 and R23

The distribution of awards (for R01s and R23s) between competing and non-competing research grant applications was as follows:

	<u>FY 1980</u> <u>Number of Grants</u>	<u>FY 1981</u> <u>Number of Grants</u>	<u>FY 1982</u> <u>Number of Grants</u>
Prior Year Commitments	679	601	680
New Research Awards	86	159	135
Renewal Awards	<u>115</u>	<u>150</u>	<u>140</u>
	880	911	955

The Institute's research grants are comprised of the following categories:

**FY 1982 Research Grants by Mechanism
(Dollars in Thousands)**

	<u>Number</u>	<u>Total Awarded</u>
Research Project Grants (R01, R23)	955	\$ 91,982
Core Grants (P30)	26	3,690
Specialized Clinical Research Grants (P50)	1	454
Research Career Development Awards (K04)	33	1,292
Academic Investigator Awards (K07)	2	66
Other Research and Research Related Grants (T09, R09, R13, S06)	13	557
Small Grants (R03)	<u>59</u>	<u>1,085</u>
Total Research Grants	1,089	99,126
Construction Grants	<u>5</u>	<u>2,000</u>
Total	1,094	\$101,126

The National Eye Institute complements its research grants with a program of institutional and individual fellowships. The purpose of the program is to equip young investigators with the skills, experiences, and insights necessary for them to embark successfully on a career in vision science, especially its clinical aspects, and other disciplines, such as the basic medical sciences, epidemiology, engineering, and biomathematics.

A total of \$3,446,000 was available for support of vision research training in FY 1982. The individual NRSA fellowship awards accounted for \$1,219,000, or 35 percent of available training funds. The institutional NRSA training awards accounted for \$2,227,100, or 65% of the program. A summary of the training program for FY 1981 follows:

VISION RESEARCH TRAINING FY 1982
(Amounts in Thousands)

	No. of Inst. Awards	INSTITUTIONAL (NRSA T32)			INDIVIDUAL (NRSA F32)			Total (T & F)	Percent Training Budget
		Pre- Doctoral	Post- Doctoral	Amount	No. of Ind. Awards	Amount			
Retinal and Choroidal Diseases	13	18	42	\$ 918*	34	\$ 549	\$1,467	42	
Corneal Diseases	6	6	29	634	8	152	786	23	
Cataract	1	0	1	-	4	68	68	2	
Glaucoma	4	0	14	226	2	39	265	8	
Strabismus, Amblyopia, and Visual Processing	10	18	23	449	25	411	860	25	
TOTALS	34	42	109	\$2,227	73	\$1,219	\$3,446	100	

* \$11,000 of this total represents NEI's co-funding of two T35's (short-term training program) under the auspices of the NIGMS for eight predoctoral positions.

The FY 1982 appropriation for the NEI included \$2,000,000 for research grants for construction of vision research facilities. Five grants were awarded.

Small Grants Program

The National Eye Institute initiated a small grants program for pilot projects in FY 1982, beginning with the October 1, 1981, application receipt date.

This is a one-year nonrenewable award intended to provide support for pilot projects, testing of new techniques, or feasibility studies of innovative and high-risk research, which would provide a basis for more extended research.

This program is designed to support:

- Clinicians with limited research experience.
- Recently trained, or less experienced, investigators.
- Investigators whose research career was interrupted and is intended to be resumed.
- Investigators changing field of research.
- Investigators at minority institutions or located in a largely non-research environment.
- Established investigators needing quick support for a pilot project.

The award may not be used to supplement support for an ongoing project.

The award will provide a maximum of \$15,000 (direct costs) for technical assistance, supplies, small equipment, and travel required by the project. The NEI expects to make approximately ten awards for each review cycle.

Applications will be evaluated with respect to the following criteria: The significance and scientific merit of the proposed project, and its characterization as an innovative and/or pilot project which provides a basis for more extended research; the methodology, including choice of experimental material; the investigator's background and training for carrying out the project; adequacy of the available and requested facilities; and the adequacy of justifications presented for budget requests.

Fifty-nine awards were made this year. It appears that the applications are being submitted by the target populations for which the program is designed. Awards are being made primarily to clinicians and nonclinicians with limited research experience.

Staff Appointments in FY 1982

The following staff appointments occurred during FY 1982:

Garrett Sanders has been appointed as the grants management specialist for the cataract and retinal-vascular disorders programs.

I. RETINAL AND CHOROIDAL DISEASES FISCAL YEAR 1982

Introduction:

The retina, the light-sensing part of the eye, is incapable of regeneration. The normal functioning and survival of retinal cells depends on a carefully controlled environment and a continuous supply of oxygen and nutrients supplied by two systems of blood vessels, one within the retina and the other in the highly vascular choroid, the tissue lying immediately underneath. Damage to the retina, interruption in its blood supply, or injury to the tissues with which it interacts, such as the pigment epithelium (a single cell layer between the retina and choroid that controls many nutritive exchanges between the blood and the retina) can lead to loss or severe impairment of vision. The retina is susceptible to injury in numerous ways, including damage from systemic disorders such as diabetes and sickle cell anemia, infection and inflammation, circulatory failure, hereditary factors, aging, trauma, and toxic and environmental factors.

Annually, 19,000 Americans become blind from retinal and choroidal diseases which cause half of all severe visual impairment in the United States; one-fourth of those affected are legally blind. One disease, diabetic retinopathy, is the leading cause of new cases of blindness in adults under age 65, and another, aging-related maculopathy, is the leading cause of new cases of blindness in people age 65 and older.

Diabetes affects many eye tissues, but its most harmful effects are on the tiny retinal blood vessels where it may trigger a series of events leading to severe and irreversible visual loss. Laser treatment can halt the progress of advanced diabetic retinopathy and forestall blindness in many cases. In some instances, when blood leaks into the vitreous humor, impaired vision can be improved through a surgical procedure called vitrectomy. Thousands of people have benefited from these advances, but the search continues for better methods of treatment and for ways to prevent or cure diabetic retinopathy.

Disease of the macula, a small area of the retina that provides sharp central vision, occurs primarily with aging, affecting about 10 million people over age 50 and frequently depriving the elderly of full enjoyment of their retirement. In May 1982, a nationwide NEI sponsored clinical trial, the Macular Photocoagulation Study, demonstrated that laser treatment can be effective in preventing severe visual loss from the neovascular type of aging-related maculopathy (characterized by the growth of abnormal new blood vessels into the macular region). The neovascular form accounts for about 90 percent of legal blindness from aging-related maculopathy and affects approximately 116,000 people in the United States. Evidence from this study suggests that 9 out of 10 of these cases of blindness could be prevented or delayed significantly by prompt laser treatment. Although these findings are impressive, the results are limited to one specific type of aging-related maculopathy, and only to patients meeting stringent eligibility criteria. More research is needed to find better ways of treating and ultimately preventing aging-related maculopathy and other macular diseases.

More research is also needed on retinal diseases that begin early in life. One of these, retinitis pigmentosa, is a disorder that often strikes young people during their critical learning years. There is neither a cure nor means of prevention of this disease which causes gradual restriction of the visual field and night blindness. Those affected face a lifetime of visual impairment and disability.

Other common and disabling retinal and choroidal diseases are retinopathy of prematurity (retrolental fibroplasia) which causes blindness in premature infants, retinal detachment, uveitis, and retinal tumors.

Intensive research over the last several years has led to dramatic improvements in diagnosis and treatment of some retinal and choroidal diseases and in certain cases to restoration of visual function. However, most of the serious diseases in this group are still poorly understood and require continued intensive research.

The following are examples of the National Advisory Eye Council's recommended Program Goals as delineated in Vision Research--A National Plan: 1983-1987, for the Retinal and Choroidal Diseases program:

- o To develop rational procedures for the prevention and cure of diabetic retinopathy, retinal degeneration, uveitis, retinal detachment, and other chorioretinal disorders.
- o To increase basic knowledge of the visual cells, the pigment epithelium, and the circulatory system of the retina and choroid, which are vital to vision.
- o To develop a better understanding of the interface and the mechanism of posterior vitreous detachment, which is an important factor in such diseases and conditions as diabetic retinopathy, retinal detachment, and ocular trauma.
- o To improve methods for tissue culture of choroidal and retinal cells for investigating normal and pathological cellular mechanisms, the molecular basis of neurotransmission, the efficacy of new forms of therapy, and the factors that promote neuronal growth and regeneration.
- o To study the function of the human retina and its neighboring tissues using noninvasive methods, both to improve diagnosis and to aid in identifying the cellular elements involved in disease processes.
- o To investigate the genetic and immunologic bases of retinal and choroidal diseases in animals, and to explore the application of innovative therapeutic approaches in these models to humans.

- o To establish a sound basis of prognosis, genetic counselling, and medical intervention by determining the etiology, natural history, and epidemiology of inflammatory disorders, tumors, and the various degenerative diseases affecting the retina and choroid.
- o To identify nutritional and environmental factors that may be toxic to the retina.

In order to meet the goals of the Retinal and Choroidal Diseases Program, research is supported within the following areas:

Vascular, Inflammatory, and Neoplastic Disorders of the Retina and Choroid

- 1) Diabetic Retinopathy, Sickle Cell Retinopathy and Other Vascular Abnormalities
- 2) Inflammatory Disorders
- 3) Tumors

Degenerative Disorders of the Retina

- 4) Developmental and Hereditary Disorders
- 5) Macular Degeneration
- 6) Retinal Detachment and Vitreous Disorders

Fundamental Retinal Processes and Retinal Disorders

- 7) Retinal Pigment Epithelium
- 8) Photoreceptors, Visual Pigments, and Phototransduction
- 9) Retinal Organization, Neurotransmission, and Adaptation
- 10) Glial Cells and the Retinal Microenvironment

Cross-Cutting Research Areas

- 11) Rescue and Regeneration of Neurons in the Optic Nerve and Retina
- 12) Noninvasive Techniques in the Study of Retinal Disorders
- 13) Toxic, Nutritional, and Environmental Disorders
- 14) Tissue Acquisition and Distribution: Human Donor Eyes and Animal Models

Recent Research Accomplishments:

The Complete Amino Acid Sequence of Rhodopsin

The primary amino acid structure of rhodopsin is being worked out actively and will probably become available in the next few years. Much research is being devoted to application of molecular biological techniques for isolation of the gene that codes for rhodopsin, which ultimately will result in

cloning of this gene. This will yield large quantities of the gene-code which, when analyzed chemically, will give an alternate approach to both the rod and cone opsin amino acid sequence. Knowledge of rod opsin sequence will give clues to the mechanism of how opsin functions in vision, while cone opsin sequence data will help our understanding of defects in color vision.

Monoclonal Antibodies in Retinal Cell Biology

Monoclonal antibodies have been produced which react with bovine rhodopsin as well as with specific retinal cell types: photoreceptors, Muller cells and ganglion cells. This technique has revolutionized the ability to produce markers against specific molecules which are characteristic of a certain cell type or a specific membrane protein. The developmental history of a membrane surface antigen gives information about the history of a specific cell type or a specific membrane protein. This is important information since specific molecules may appear at different times on specific cells of the retina and may not be present during the entire life of the cell. This would be particularly important in studying the formation of synapses between cells using tissue culture techniques. It may be possible to identify cell molecules which regulate the process of cell communication and thus gain information about how connections are made from photoreceptors to second and third order neurons. Monoclonal antibodies made against rhodopsin have yielded information on how this molecule is oriented in the photoreceptor membrane. Antibodies specific to other photoreceptor molecules will yield information on their role in the transmission of information that begins with the response of rhodopsin to light and ultimately results in membrane hyperpolarization. Identification of molecules which are present in normal retinas but absent in dystrophic retinas may give clues to molecular abnormalities which form the basis of retinal disease.

New Techniques for Studying Transduction at the Cellular Level

Two new microelectrodes have been developed which will help in understanding events important in phototransduction. The first type is a cyclic GMP-specific electrode which can measure very small changes in the level of cyclic GMP upon light exposure of intact rod outer segments. Development of electrodes specific for molecules involved in phototransduction are technologically difficult because an enzyme (for example, phosphodiesterase) must be immobilized in a gel and yet be able to react chemically with its substrate (cyclic GMP). Additionally, the tip of the electrode must be so constructed as to completely engulf the outer segment of a rod cell. This approach to study of the fundamental events surrounding transduction has the advantage in that it is possible to study a single cell's response to light.

Another microelectrode has been developed which can seal or clamp a small portion of a cell membrane to its tip. It is then possible to present the two sides of the membrane with various molecules under varying conditions of light and electrical stimulation. Information from experiments using this patch-clamp technique may give clues as to how physiologically important ionic currents are generated.

Vitamin E, Antioxidants, and Oxidative Damage

Much evidence leads to the concept that oxidative reactions in the retina lead to various observed degenerative changes. A long-range goal is the management of eye disorders that are secondary to light-induced or drug-induced formation of lipid peroxides.

The oxidation of membrane lipids from rod visual cells is thought to contribute to formation of lipofuscin or "age-pigment" in the retinal pigment epithelium. It is likely that this pigment accumulates with age because outer segments contain highly unsaturated membrane fatty acids and a high oxygen tension exists across the pigment epithelium and outer segments. Indeed, outer segment lipids have been shown to be susceptible to auto-oxidative damage, the reaction products probably becoming engulfed by the pigment epithelium in the normal process of phagocytosis.

The retina contains significant levels of the antioxidants Vitamin E and selenium. The exact role of these natural antioxidants is not clear but deficiencies of these two compounds lead to accelerated pigment accumulation in the pigment epithelium. Dietary deficiency of Vitamin E can produce central retinal degeneration in monkeys. The element selenium is a component of the enzyme glutathione peroxidase which is found in high levels in the pigment epithelium. This probably indicates that anti-oxidative mechanisms are important for normal retinal function. Rats maintained on diets deficient in anti-oxidants have a reduced rate of phagocytosis by the pigment epithelium. This is partly accounted for by photoreceptor cell loss and accumulation of cellular debris between the photoreceptor outer segments and pigment epithelium. This kind of phenomena is observed in hereditary retinal degeneration in the RCS rat where an apparent retinal pigment epithelium defect causes accumulation of membrane debris at the photoreceptor/pigment epithelium interface.

Light Damage

It is known that high levels of light and oxygen can be toxic to the retina. The question that has arisen is whether or not normal levels of light can over long periods of time in some individuals cause damage to the retina. Short wavelength light (blue-green) is more energetic than long-wavelength light (red-yellow). Recently it has been shown that when monkeys are exposed to high levels of blue light there is irreversible damage to blue-sensitive cones. Intermittent green light exposure produces functional

loss of green cones which is recoverable over a period of weeks. This information, combined with knowledge that primate retinas exposed to blue light causes hypopigmentation of the pigment epithelium points out the potential toxic effects of light.

There are a number of devices which expose the retina to relatively light levels, for example laser photocoagulators, lasers for holography, and intense illumination for eye microsurgery. More information is needed on the mechanism of light-damage to the retina as well as minimum energy of light required to produce lesions.

Vitamin A

Vitamin A is crucial to light transduction in the retina because of its light-absorbing role when bound to the protein rhodopsin. Until recently the translocation and uptake of Vitamin A both within and between the pigment epithelium and the receptor cells has not been understood. Investigators have been studying the roles of specialized proteins involved in transport and uptake of Vitamin A. An 11-*cis*-retinal binding protein, uniquely localized to retina and preferring 11-*cis*-retinal, is being characterized.

Recently a protein has been found in the subretinal space having properties of an interphotoreceptor retinol-binding protein and functioning as a Vitamin A transporter between neural retina and pigment epithelium. Two different transport proteins have been demonstrated in rabbit retina subretinal fluid, one of which shows differential binding of retinol in light versus the dark perhaps hinting at its physiological function.

Fluid Transport Across the Retinal Pigment Epithelium

The pigment epithelium provides a major transport pathway between the choroidal blood supply and the neural retina. Recent studies in the frog have for the first time shown that the direction of net fluid movement is from the retina to the choroid. Net transport is significantly decreased by dibutyryl cyclic AMP and isobutyl methylxanthine, a phosphodiesterase inhibitor. The implication is that this transport mechanism may be important in controlling the volume of the subretinal space. This may be an important factor which contributes to the adhesive forces by which the retina remains in close apposition to the retinal pigment epithelium. These studies may provide new clues concerning the photogenesis of retinal detachment.

Human Donor Eyes

Timely access to donor human tissue is needed in order to confirm and expand findings in animals, and for studies in which there is no other suitable tissue. Recently a novel preparation has been developed

which allows multiple samples of tissue to be taken from single human retinas for combined morphological, biochemical, and tissue culture studies. This is a powerful approach which allows the localization of specific molecular events to specific retinal regions and cell types. The gathering of basic normal data is underway and investigation of diseased human retinas show biochemical abnormalities such as differences in esterification of 11-cis retinal and protein synthetic ability of photoreceptor cells.

Pattern Evoked Retinal Response

It has been generally accepted that the inner retinal layers do not contribute to ERG activity. In patients with optic nerve atrophy, for example, the ERGs elicited by a diffuse flash are not at all affected. However, it was recently demonstrated that in the cat, at least, optic nerve section obliterates pattern evoked retinal responses (PERR), while diffuse flash ERGs remain normal. Thus, the PERR, unlike the diffuse flash ERG, appears to be dependent on the integrity of the inner retina. Current research is attempting to answer the question of where and how this response is generated in the retina. The answers should provide a powerful new tool for understanding inner retinal pathologies.

Aging-Related Maculopathy (Senile Macular Degeneration)

Aging-related maculopathy is the leading cause of severe visual loss in people age 65 and over. About 100,000 Americans are legally blind as a result of this disease and over 15,000 new cases of this disease are reported each year. A randomized, controlled clinical trial supported by the National Eye Institute to study treatment of this disease has shown that for the so-called neovascular form of aging-related maculopathy, visual loss can be significantly prevented through the use of argon laser photocoagulation. This study, called the Senile Macular Degeneration Study (SMDS), shows that photocoagulation of new blood vessels in aging-related maculopathy patients greatly reduces the risk of severe visual loss. In order to be enrolled in this study patients had to meet certain eligibility criteria. These included: presence of drusen, evidence of a choroidal neovascular membrane between 200 and 2500, μ m from the central fovea, best corrected visual acuity of 20/100 or better, no other interfering ocular disease, and age 50 years or older. Eligible patients were assigned randomly to a "treatment" group or a "no treatment" group. After 18 months patients are being recruited, but follow-up studies continue so that long-term assessment of the treatment can be made. Because of the significant treatment benefit resulting from this study, a scientific paper was immediately published in the June, 1982, issue of Archives of Ophthalmology in order to inform all primary care physicians as quickly as possible. A natural history study and the treatment trial for the presumed ocular histoplasmosis syndrome will continue.

The Senile Macular Degeneration Study (SMDS) demonstrated the effectiveness of photocoagulation therapy with the argon laser in reducing loss of vision due to neovascular aging-related maculopathy. However, many patients with this disorder are not eligible for argon laser treatment because the area to be treated is too close to the center of the retina (the fovea), where argon laser treatment would accelerate the loss of vision. Recently, a krypton laser has become available which may be safer to use for treatments closer to the fovea. The Krypton Photocoagulation Study has been initiated as an extension of the SMDS to determine the effectiveness of krypton laser treatment in eyes that are not suitable for argon laser treatment.

Psychophysics and Electrophysiology in Evaluation of Patients With Diabetic Retinopathy

Retinal defects in early stages of diabetic retinopathy (prior to clinically apparent retinal vascular changes), may have been underestimated previously. Most emphasis has been placed on secondary retinal vascular changes because they are easily observed via ophthalmoscopy, fundus photography, and angiography.

The ERG, EOG, dark adaptation, contrast sensitivity, perimetric studies, and Stiles-Crawford testing may give early clues to retinopathy. It has been shown that in adult diabetics there is a significant change in blue cone sensitivity which parallels progress of the disease. This suggests that chromatic sensitivity loss may occur in the early stages of diabetes and this may precede retinopathic abnormalities.

New Techniques for Detecting Ocular Tumors

The angiogenic capacity of aqueous humor of human eyes forms the basis of a new *in vivo* diagnostic assay as a diagnostic marker of eye malignancy. This test is based on endothelial cell migration. The aqueous humor from patients with malignant eye tumor shows an increased endothelial cell migration using an assay system consisting of glass coverslips plated with colloidal gold. This may be an effective means for the early diagnosis of ocular tumors.

Angiogenesis

Retinal capillaries are of current research interest because in diabetic retinopathy the pericytes (mural cells) which surround the blood vessels degenerate, but the endothelial cells remain and subsequent blood vessel proliferation ensues. Recently it has been possible to grow long-term, pure cultures of retinal capillary endothelial cells and pericytes. The availability of cultures of individual retinal capillary cell types will be useful in further studies of the biochemistry, physiology and growth potential of these retinal blood vessels. Thus, recent experimental results show that extracts of adult retinas from various mammalian species

are potent stimulators of angiogenesis. There is good biochemical evidence that these chemotactic factors are proteins. Understanding retinal capillary cell biology is important to progress in management of retinal vascular disease, especially diabetic retinopathy.

Treatment of Uveitis

Uveitis causes about 10% of the cases of visual impairment in the United States. The cause appears to be immunogenic or autoimmune in nature. Recently it has been found that an antigen isolated from mammalian photoreceptors, the S-antigen, can induce autoimmune inflammatory response. When S-antigen is injected into rhesus monkeys, ocular inflammation ensues in a matter of weeks, characterized by focal and deep retinal lesions. Photoreceptor cell layer loss results as a consequence of this treatment and points to the specificity of the immune response. Cyclosporin A, obtained from fungal extracts, is beneficial in preventing or at least modulating the uveitis in the monkey experimental model. This drug may prove to be an important tool in altering severe ocular inflammatory reactions.

Research Needs:

Two clinical trials, the Diabetic Retinopathy Vitrectomy Study (DRVS) and the Early Treatment of Diabetic Retinopathy Study (ETDRS), were initiated as a result of the success of the nationwide Diabetic Retinopathy Study (DRS). The recently announced success of the ETDRS gives further evidence of the way clinical trials can pave the way to prevention of visual disorders. Support is required to initiate new clinical trials as promising treatments become available, for example, as with testing the efficacy of rigorous blood sugar control to prevent complication of diabetic retinopathy.

Regarding ocular melanoma, important questions as to the epidemiology, natural history, optimal therapy, and cytologic classification need to be answered. The most promising approach to the resolution of these questions would be through basic biologic studies on the genesis of ocular tumors. Prospective studies of patients with uveal melanoma are needed, which will gather much needed diagnostic, epidemiologic, and treatment data in accordance with standardized protocols.

Information is needed on the immunology of retinoblastoma so that earlier diagnosis can be made. Further studies are needed to determine the role of viruses as a cause of retinoblastoma. Molecular and immunologic studies are needed to look for identifying characteristics of viruses which may be present in these tumors. Animal models of these tumors would be useful in determining the role viruses play in initiating this disease.

Further advances in the use of noninvasive techniques to assess retinal function in the early stages of disease process are needed so that early detection can be made for certain hereditary disorders. Further refinements in electroretinographic and psychophysical testing, as well as in depth examination of, for example, the cone b-wave of the electroretinogram, will give earlier and more refined information on the early stages of diseases like retinitis pigmentosa. The search for animal models of hereditary retinal degenerations should continue so that we can better describe the natural history of these conditions using noninvasive techniques.

More research is needed on photoreceptor cell metabolism, especially the means by which visual cells replace certain components since this process is clearly vital to the cell's survival. In this regard, the potential damaging effects of light on retinal cells, as well as the influence of oxygen in hastening the destruction of cell membranes, need to be clarified.

Much information has been gained on how cells of the retina are connected to one another so as to provide an electrical network of forward and feed-back loops. The biochemical transmitters which retinal cells use to communicate with one another need to be identified, as well as the specific effects of neurotransmitters on post synaptic cells.

II. CORNEAL DISEASES FISCAL YEAR 1982

Introduction:

Diseases of the cornea constitute an important cause of blindness and visual impairment and an immense economic burden on society.

Each year, more than 2 million cases of corneal disorders and at least 1.7 million injuries to the cornea are recorded. These cases constitute 62 percent of the total incidence of all acute and chronic afflictions of the eye, accounting for more than 100,000 hospital days and \$12 million in surgical costs. Discounting refractive error, corneal problems require about 10 million annual office visits or one-third of all visits for medical and surgical eye treatment, and consume a major portion of the eye care dollar. Although corneal disease produces about 6 percent of all legal blindness (less than 20/200 vision in both eyes) in the United States, it is the primary cause of bilateral blindness in the rest of the world.

In this report, a few of the research areas presently exhibiting high scientific activity will be considered in relation to the research priorities for the Corneal Diseases program as outlined in the 1983 report of the National Advisory Eye Council, Vision Research--A National Plan: 1983-1987. These areas are: contact lens effects on refractive error, epikeratophakia, corneal endothelial transplantation, corneal wound healing, corneal keratinization, transparency of the cornea, tear secretion dynamics, and typing of herpes simplex viruses.

Program Goals

- o To determine the neuronal and viral factors contributing to the latent period of herpes infection, correlate the biological and biochemical characteristics of various herpes strains with the disease characteristics they cause, and determine viral latency patterns and mechanisms which trigger viral reactivation and shedding.
- o To continue to develop specific antiviral drugs for acute and recurrent corneal infections.
- o To evaluate the normal immunologic mechanisms of the ocular surface and its role in disease.
- o To study corneal epithelial defect formation, occurrence, and persistence, and relationship with neural supply.
- o To study biological effects of existing and proposed procedures and materials for surgical or lens correction of refractive error.

- o To develop methods for in vivo assessment of corneal metabolism and nutrition and compare the functioning of normal and diseased corneas from animal models and man.
- o To evaluate changes in the biochemistry of the corneal stroma with disease and aging.
- o To study the basic mechanisms responsible for development of corneal dystrophies.
- o To develop methods to stimulate repair of the corneal endothelium, in vivo, especially in primates.
- o To improve corneal transplant procedures using cultured endothelial cells.
- o To improve understanding of the biochemistry underlying the corneal response to injury and wound healing.
- o To determine which of several steroids is most effective in preventing corneal tissue destruction and promoting wound healing.
- o To determine the importance of specific immunological factors in corneal transplantation and develop and test drugs which can modify or eliminate immune reactions to transplanted tissue.

CORNEAL DISEASES

Subprogram

A. External Ocular Infections and Inflammatory Diseases

1. Herpes Simplex
2. Herpes Zoster
3. Adenovirus and Enterovirus
4. Bacterial and Fungal Keratitis
5. Chlamydial Keratoconjunctivitis
6. Chronic Blepharitis
7. Other Infections

B. Ocular Surface Problems

1. Tear Film and Its Abnormalities
2. Ocular Surface Disorders
3. Drug Delivery and Toxicity

C. Refractive Problems and Contact Lenses

D. Corneal Edema, Endothelial Dysfunction, Dystrophies, and Inherited Diseases

1. Endothelial Tissue Culture, Replacement, and Repair
2. In Vivo Evaluation of Corneal Epithelial and Endothelial Membrane Function
3. In Vivo Morphologic Evaluation--Specular Microscopy
4. Endothelial and Epithelial Transport Processes
5. Stromal Swelling and Transparency
6. Normal Corneal Development
7. Corneal Dystrophies, Inherited Disorders, and Developmental Anomalies

Subprograms/Areas

- A. Corneal Transplantation and Stromal Wound Healing
1. Inflammation and Repair
 2. Corneal Transplantation

Recent Research Accomplishments

Contact Lens Effects on Refractive Error - Clinical Trial

Since the development of contact lenses for correction of refractive error, unanticipated and undesirable changes in corneal curvature have often been observed clinically in a variety of patients fitted with hard contact lenses. Such observations have prompted certain clinicians in recent years to induce these changes purposely by special lens fitting techniques, in an attempt to reduce or alleviate myopia. This procedure, often referred to as orthokeratology, has sparked a great deal of controversy in the ophthalmic community because it represents a departure from the traditional viewpoint that the goal of successful contact lens fitting was to avoid any corneal change.

In order to resolve the controversy about orthokeratology the National Eye Institute provided financial support to Polse and co-workers at the University of California at Berkeley in June, 1976, for development of a protocol which outlined the conduct of a randomized, controlled clinical trial. This trial was designed to assess the corneal and visual changes resulting from lens designs used in orthokeratology as compared to the effects of contact lenses which were fitted in a standard clinical manner. A manual of operations for the trial was completed in February, 1978, and the recruitment of subjects began in March of that year for a three-year period.

Data were gathered in a double-masked method and subjects were randomly allocated to either treatment or control groups. In all, 31 treatment and 28 control subjects were assessed after an average of 434 days of contact lens wear for comparative changes in: (1) visual acuity, (2) refractive error, (3) corneal curvature, (4) corneal thickness, (5) endothelial cell density, (6) duration of effect, and (7) predictive factors.

Upon completion of the trial the following major conclusions were derived from the study:

1. It is possible to reduce myopia an average of 1.0 D by wearing appropriately fitted contact lenses.
2. The change is not permanent and requires some form of contact lens wearing regimen to maintain the therapeutic effect.
3. Vision without contact lenses is variable and can fluctuate from day to day making it difficult to predict the level of vision during periods of non-contact lens wear.
4. Methods for maintaining stable vision during periods of non-contact lens wear need to be developed before orthokeratology will be a clinically appealing therapeutic treatment for myopia.

These findings plus all pertinent trial data will soon be published in a series of three papers in the journal Archives of Ophthalmology.

Corneal Endothelial Transplantation

There has been an increased desire on the part of ophthalmic surgeons in recent years to find a suitable immediate approach to the removal and replacement of a diseased endothelial layer of the cornea, if necessary, during penetrating keratoplasty. Although initial experimental attempts at heterologous endothelial transplants in lower vertebrate species have met with good success, progress in development of a clinically suitable transplantation technique has been slowed by several technical difficulties, even when cells of the corneal endothelial layer of the donor animal species resemble those of the human in their biological characteristics.

One shortcoming of the present experimental approaches to transplantation has been the long time required to reconstitute a new endothelial layer through seeding of the appropriate cultured cells from the donor animal onto the recipient Descemet's membrane in the laboratory. Alvarado and his colleagues at the University of California at San Francisco have observed that the seeding process requires over an hour to complete when a time less than fifteen minutes would be more suitable for a delay in surgery.

Another aspect of such techniques which has created difficulties but which appears recently to be lending itself to some resolution is the method employed for denuding the Descemet's membrane of the recipient cornea of its own endothelium. Use of alkali to effect this process resulted in much inflammation, destruction of bovine cells being implanted, and the formation of permanent retrocorneal membranes. When Triton-X, a non-ionic detergent, was substituted for ammonium hydroxide as a denuding agent, no inflammation was observed in the grafted animal and only transient retrocorneal membranes were observed in some cases. In addition, no evidence of a graft rejection reaction was observed with the use of this

Since the endothelial cells of the cat cornea, like those of the human cornea, do not readily undergo mitosis, transplantation investigators in the near future will continue to use this animal as a recipient species for endothelial grafts to test a variety of donor cells, e.g., human vascular endothelial cells, and for resolving problems in achieving proper cell density through tissue culture. Only when suitable progress has been made with the cat model will a full commitment be made by investigators to substitution of the more costly primate as a recipient species.

Epikeratophakia

Several of the refractive keratoplasty procedures as currently employed have marked limitations for application to the correction of myopia. The complexity of these surgical techniques as developed and perfected by Barraquer precludes their use by large numbers of ophthalmic surgeons. A modified surgical procedure termed "epikeratophakia" theoretically meets most of the objections raised against such procedures.

Epikeratophakia involves the suturing of preserved, pre-shaped corneal tissue through prior lathing atop a recipient cornea from which the epithelium has been surgically removed. This maneuver obviates the risks and difficulties of lathing corneal tissue in the operating room during surgery and avoids the potentially hazardous procedure of lamellar keratoplasty. Epikeratophakia appears to be safe, technically simple, has good optical potential and to be generally applicable and reversible.

Epikeratophakia is theoretically applicable to all classes of aphakia patients, but there are several categories of patients for whom this may prove to be the best available therapy: 1) adult patients who have had unilateral cataract surgery but who cannot tolerate contact lenses and are not candidates for secondary implantation of an intraocular lens, 2) patients with moderately advanced keratoconus who can no longer tolerate contact lenses, 3) children and adults with unilateral high myopia who cannot tolerate contact lenses, and 4) infants and children with traumatic and congenital cataracts. This last category is especially important because there currently is no satisfactory way of rehabilitating vision in these children. Although cataracts are easily removed, if vision cannot be restored to the aphakic eye in children with reasonable speed, disuse results in amblyopia and eventual loss of sight. The epikeratophakia graft has the advantage of being permanent, which frees the parents of such children from the burdens of maintaining contact lens correction in the eye, and allows more attention to be paid to the patching schedule that is imperative in amblyopia therapy.

Research on the long-term stability and efficacy of the epikeratophakic graft has been carried out on monkeys housed at the Delta Regional Primate Research Center by Safir and his colleagues at Louisiana State Medical Center at New Orleans. This team has been investigating the use of xenograft corneas for donor tissue and whether alloplastic materials can be used in conjunction with these grafts. The latter research project is specifically directed toward achieving greater than 30 diopters of correction for use with certain young aphakic patients in order to prevent the onset of amblyopia.

Keratinization Assay for Xerophthalmia

A common symptom observed in malnourished children suffering from vitamin A deficiency throughout the world is keratinization of the ocular surface epithelium. By use of monoclonal antibodies to individual keratins, investigators at the John Hopkins University have determined that two specific markers, i.e., keratins of molecular weights 65,000-67,000 (65-67K) and 56,000 daltons (56K), respectively, accumulate in the ocular surface epithelium in vitamin A deficient rabbits. Keratins are a family of water-insoluble proteins which form 10 nm tonofilaments in skin and in the ocular surface epithelium. While 65-67K and 56K keratins are normally present in skin, they are not seen in the ocular epithelial layers.

If similar differences occur in the pattern of keratins present in normal and vitamin A-deficient human skin and ocular surface epithelium, preparation of the corresponding monoclonal antibodies against such human keratins may lead to development of an important field assay for early detection of vitamin A deficiency using cells obtained from conjunctival epithelium.

Cell Motility and Adhesion in Corneal Wound Healing

A procedure has been developed by Gipson and her colleagues at the Eye Research Institute in Boston for obtaining intact viable sheets of corneal epithelium from rabbit corneas by use of Dispase II, a neutral protease derived from the microorganism *Bacillus polymyxa*. In corneal buttons, incubated for 1 hour at 35°C with this enzyme, the epithelial layer separates at the level of the hemidesmosome-basement membrane attachment. This research has revealed considerable information about hemidesmosome formation:

- 1) Freed epithelial sheets form new hemidesmosomes rapidly when placed on denuded corneal stromas with intact basement membranes.
- 2) Significantly, hemidesmosomes form over anchoring fibrils present beneath the basement membrane. These fibrils seem to be the site of initiation of hemidesmosome formation.
- 3) Hemidesmosomes will not form when epithelial sheets are placed over Descemet's membrane or lens capsule, which indicates that different ocular basement membranes have unique molecular compositions.
- 4) These organelles form when rabbit epithelial sheets are placed on denuded human or rat stromas.
- 5) Calcium ions are required for the formation and maturation of hemidesmosomes.

The important knowledge gathered from such basic studies as these will ultimately be transferred to the clinical setting and may cause substantial modifications in presently employed transplantation procedures which involve the cornea.

In Vision Research--A National Plan: 1983-1987 the Corneal Diseases planning panel identified the study of normal epithelial healing, including mitosis, sliding, and adhesion and the effect of mitogens and growth factors on these processes as representing one of the major research objectives of the Program.

Corneal Transparency and Level of Sulfate Donor

The normal mechanism by which transparency occurs in the developing cornea is not understood. Studies using the chick as a model have recently shown that the corneal transparency in this animal begins to develop at day 14 of embryogenesis and reaches the adult level on day 19, and that the process is influenced by the level of thyroxine present.

The degree of sulfation of keratan sulfate, the major glycosaminoglycan of the cornea, also increases during the time of rising corneal transparency beginning on day 14. Simultaneously, the synthesis of the sulfate donor, 3' phosphoadenosine-5' phosphosulfate (PAPS), but not the enzymes that transfer the sulfate moiety to the glycosaminoglycans, begins to increase and reaches a peak on day 16. Thus, the increase of PAPS in chick corneal tissue is correlated with a crucial event in the initiation of the development of transparency. It will be interesting to observe whether embryonic corneas from other vertebrate species demonstrate similar results and whether alterations of tissue levels of PAPS are observed in conditions which are associated with congenital corneal clouding.

Tear Secretion Dynamics

A new, more accurate Schirmer-type of test that is still fairly simple to use has been devised by Holly and co-workers at Texas Tech University. This test provides physiologically meaningful information such as the magnitude of the initial and final tear secretion rates and the secretion rate decay coefficient. Such information should be useful in the future for detecting marginal dry eye conditions and for predicting poor contact lens tolerance.

Typing of Herpes Simplex Viruses

A rapid serological technique has been developed by Cleveland and his associates at the University of California at San Diego which can accurately identify a herpes simplex virus isolate as type 1 or type 2. The assay employs filter paper disks to immobilize viral antigens present on the membranes of infected cells. The antigens are identified by a reaction with ¹²⁵I-labelled staphylococcal protein A following incubation of infected cells with cross-absorbed herpes simplex virus typing serum. The serological technique can be rapidly executed (in less than 2 hours), requires minute quantities of typing sera, and can be easily performed with cells from a single infected roller tube culture. Moreover, the method is particularly suited to the simultaneous analysis of many specimens and is amenable to automation. If this assay can be adapted to the consistent detection of viral antigen on ocular epithelial cells collected from eye swabs, it will find particular use in scanning neonates born of mothers with suspected genital herpes.

Future Needs

To further our understanding and clinical management of recurrent ocular herpes simplex disease, it is especially important that its pathogenesis and especially the immunologic mechanisms attending it be better understood. A valid animal model of recurrent disease is needed, one in which the beneficial and deleterious aspects of the ocular immune response could be differentiated. Genetically defined mouse strains, in which lymphocyte subsets and histocompatibility antigen control mechanisms are becoming well defined, may be the best model system for immediate use. Monoclonal antibodies to herpes virus antigen, either used alone or conjugated to an antiviral drug, should be evaluated in a suitable animal model for their clinical potential. Long term longitudinal studies should be initiated to follow the clinical course of herpes simplex in order to determine quantitatively the measurable parameters of the disease, especially correlations of virus strain and original type of infection, recurrence patterns, types of immunoglobulins produced, and cellular populations involved in the cytotoxic immune response. Better definition of the interactions of the ocular immune response will also be of benefit in furthering our understanding of the mechanisms of corneal transplant rejection and how they might be overcome.

Much remains to be defined about tear film in health and disease. The relationship between the film constituents and the physiology of normal and diseased corneal epithelium and conjunctiva needs to be defined. Microassays should be developed or exploited to quantitate tear film constituents in order to improve tear replacement therapy and to protect the ocular surface during contact lens use.

Of especial importance are new studies on the cellular biology and physiology of the corneal epithelium, its disease and healing processes. Studies are needed on the genesis of corneal epithelial defects and their relationships to neural, hormonal, and autacoidal agents. The factors which prevent epithelial cells from spreading and adhering to the basement membrane must be defined. An appropriate animal model would permit more precise examinations of ocular surface healing processes. Animal studies are also relevant to ascertaining the benefits and risks of using contact lenses to treat corneal disease. Also needed are improved methods of measuring parameters of corneal health when contact lenses are worn for extended periods of time.

Similarly, in vitro studies are essential to understanding the biology of the corneal endothelium, particularly with respect to new methods of transplantation. The seeding of homologous or heterologous cultured cells on a membranous support (or the patient's own denuded corneal button) holds promise as a substitution for use of donor corneas. Many technical factors must be clarified before use of cultured cells will be feasible, including determining how cells can be attached to a membrane and corneal stroma within the time patient is in surgery, the immunologic aspects of host response to cultured donor cells, and if other types of endothelial cells can substitute for corneal cells. Of possibly greater importance is the search for means by which the patient's own endothelial cells might be stimulated to proliferate and repair endothelial defects.

The physiologic and metabolic requirements of the endothelium, and how epithelial cell function affects them, remain to be elucidated. To aid in evaluations of corneal endothelial function, improved instruments for specular microscopy are needed, particularly a wide-field scanning and recording device.



III. CATARACT FISCAL YEAR 1982

At present, surgery to remove the opaque lens is the only effective way of treating cataract. Surgical techniques developed over the past 25 years have made cataract extraction one of the safest and most successful of any major operation. Although most cataract patients are grateful for their restored visual acuity, all naturally would have preferred to have avoided cataract altogether. For this reason the National Eye Institute appropriately devotes most of its funding in the Cataract Program to research aimed at developing means of preventing or slowing cataract development or for treating it nonsurgically.

The Goals of the Cataract Program are as follows:

- o To find means of preventing or slowing cataract development.
- o To determine the causes and mechanisms of cataract formation.
- o To understand the development, biochemistry, and biophysics of the normal lens.
- o To evaluate the safety and efficacy of methods of cataract extraction.
- o To evaluate new methods for correcting optical problems that follow surgery.

The Subprograms of the Cataract Program:

1. The Normal Lens
2. Epidemiology of Cataracts
3. Senile Cataract
4. Diabetic and Metabolic Cataract
5. Nongenetic Congenital and Genetic Cataract and Dislocated Lenses
6. Cataract Induced by Environmental and Toxic Effects
7. Treatment of Cataract and Correction of Aphakia

Recent Accomplishment

(1) The Cooperative Cataract Research Group (CCRG)

The Cooperative Cataract Research Group consortium is composed of twenty three institutions nation-wide, eleven of which are currently funded through a grant from the National Eye Institute (NEI).

The scientific momentum achieved by the entire consortium during the past year was truly remarkable. The individual reports from each center document in considerable detail the advances made in many areas

of human cataract research. Many of the publications are the results of collaborative efforts within the consortium and a few have involved international collaboration. The efficient use of NEI funds by the small group of scientists working on an extraordinarily complex problem is evident.

The CCRG system of classifying cataractous changes in the human lens has proven to be most useful. A basic feature is the establishment of standardized photographic procedures for the classification of human lens opacities. These procedures make possible the correlation of medical histories with specific classes of human opacities. These procedures also make possible the study of the morphology and chemistry of human cataract in a rational way. The morphology of specific individual opacities, as seen in the freshly extracted human lens can be correlated with their ultrastructure by scanning and transmission electron microscopy and with their chemical composition through energy dispersive x-ray analysis (EDXA). EDXA in conjunction with scanning electron microscopy (SEM) was used to analyze a group of human cataractous lenses that contained small, scattered, localized (punctate) opacities. These areas of opacification were clearly seen in the CCRG stereo slides made of each lens while fresh. EDXA analysis of these opacities showed a high concentration of phosphorus and little or no sulfur, while the normal appearing lens fiber immediately adjacent to an opacity, in contrast, contained very little phosphorus, but large amounts of sulfur.

The CCRG classification system has led to the observation that although nuclear color is positively associated with age, it is not associated with the type or extent of cataract formation and should not be used to infer either the type or extent of opacification present.

Dr. S. Lerman, a member of the CCRG, had demonstrated the feasibility of measuring age-related lens fluorescence and density changes *in vivo* with a modified Scheimpflug UV-visible slit lamp densitograph. Over 300 normal human eyes (1st to 9th decade) were followed for at least one year. Data shows a good correlation with *in vitro* lens fluorescence measurements and indicates that molecular changes can be monitored in the normal lens months to years before visible opacification becomes manifest.

(2) Diabetic Cataracts

Sorbinil, an aldose reductase inhibitor, prevented cataracts in rats fed a 50% galactose diet for 8 months. A similar experiment with diabetic rats indicated that Sorbinil was able to prevent the accumulation of sugar alcohol in the lens for over 12 months, and these rats did not have cataract development or any lens changes during this time.

A workshop on aldose reductase sponsored by the National Eye Institute was held in March, 1982, at the National Institutes of Health. The participants summarized and discussed recent finding on the properties of aldose reductase, its activity in different tissues of the body, its possible roles in the various complications of diabetes, and its control by specially designed aldose reductase inhibitors

A highly significant workshop presentation showed that aldose reductase inhibitors are effective in reversing certain abnormalities (neuropathy) in diabetic patients. This finding is the first evidence in humans that aldose reductase inhibitors may alter the course of diabetic complications in tissues outside of the eye. The next phase will be to plan for a clinical trial of Sorbinil in diabetic retinopathy.

The importance of basic laboratory work directed to understanding the basis for cataract formation, which in turn led to treatment modalities, has been demonstrated in these studies of diabetic cataracts. This clearly shows that once a cause of a disease is understood a rational approach to treatment is possible.

(3) Lens Proteins

Another area of cataract research where inroads in understanding the processes that lead to opacification has been uncovered in the study of proteins. For many years it has been suspected that the aggregation of proteins is responsible for light scattering and opacification. Significant advances have been made into the detailed nature of protein changes that lead to aggregation. The light scattering elements of cortical cataracts are the large intercellular clefts and areas of fiber cell breakdown. High molecular weight aggregates present in the cortex may also play a role. The greatly increased level of high molecular weight aggregates probably accounts for the light scattering in nuclear cataracts.

The mechanism(s) that lead to oxidative damage of the lens remains to be elucidated. A prime factor appears to be sunlight acting via ambient ultraviolet light radiation (300-400 nm). This radiation is capable of generating fluorescent pigments via a photochemically initiated free radical mechanism involving tryptophan and other aromatic amino acids as the absorbing chromophores. The accumulation of the pigmented species accounts for the increased yellowing of the lens nucleus with age and the increase of non-tryptophan fluorescences. The accumulation of the fluorescent pigment would serve to cross-link proteins into large aggregates.

Evidence for free radical mechanisms in fluorophor production in the aging human lens has been obtained. The free radicals are produced by a biphotonic process in which triplet tryptophan is a photosensitizer transferring energy to any receptor in the human lens. Alternately, the damaging effect of UV light may be due to the generation of H_2O_2 or to production of neutral tryptophan residue and a superoxide radical. An accumulation of H_2O_2 in the lens can lead to the oxidation of protein and glutathione-SH groups to GSSG, protein glutathione mixed disulfides, and protein disulfides.

The role of singlet oxygen in the in vitro cross-linking of lens crystallins in a photodynamic system consisting of visible light, a photosensitizer, and oxygen has been demonstrated. Bovine and human

crystallins become cross-linked with the generation of blue fluorescence typical of that seen in human cataractous lens. Appropriate conditions for the generation of singlet oxygen exist *in vivo*, including photosensitizers such as riboflavin and N-formyl kynurenone. Whereas the singlet oxygen may be counteracted in the cortex, the resultant photoxidized protein molecules accumulate in the nucleus where antioxidants such as glutathione are present in much lower concentration.

(4). Membrane Proteins and Lens Cytoskeleton

About 90% of the total mammalian lens protein is composed of crystallins. The noncrystallin proteins are mainly found in the albuminoid fraction or water insoluble fraction. Until recently this fraction had been largely ignored in lens research. The water insoluble fraction can be further fractionated by 6 to 8 M urea extraction into urea-soluble and urea-insoluble fractions. The first fraction contains mainly constituents of the intercellular matrix, whereas the latter comprises the intrinsic plasma membrane proteins.

After preliminary studies on calf lens plasma membrane, it seemed necessary to investigate systematically those proteins that are extrinsic or intrinsic parts of the lipid bilayer of lens cell membranes. It was suggested that abbreviation "MP" should stand for membrane protein followed by the apparent molecular weight in kilodaltons (K). The principal fiber plasma membrane protein with a molecular weight of about 26,000 is indicated by MP 26. Another important lens membrane component, typical for both epithelial and fiber cell membrane, is MP 34. There are two additional polypeptides in the molecular weight range of 42K and 55K. The 42K polypeptide has been identified as actin.

The cytoskeleton of the epithelial cells consists of microtubules, intermediate filaments, and actin filaments. In the anteriorly flattened accommodation lenses of man, squirrel, and frog, the filaments are scattered in the cytoplasm. Microtubules are present only in the superficial cortical fiber cells, decrease in number in the deeper fibers, and are absent from the nuclear fiber cells. However, in the newborn human lens, and in the chick lens at hatching, intermediate filaments are present in cortical and nuclear fiber cells. Since filaments are present in the superficial fiber cells and the adult lens, their absence from the nuclear cells must be due to degradation with age.

In the squirrel and infant human lens, a heavy accumulation of filaments is present on either side of the epithelial-fiber cell junction, especially on the cytoplasmic side of fibers. These filaments form a three-dimensional lattice and some are associated at one end with membrane. The lattice is less dense along the fiber cells not abutting on the epithelium. In deeper anterior fibers the filaments are located parallel to the membrane.

Another element of the fiber cell cytoskeleton, represented by beaded filaments, was described by many investigators. In chick and frog lenses these structures, referred to as chains, consist of globular particles, arranged as a filamentous backbone. In the mammalian lens the globular particles are similar to alpha-crystallin. The chains appear as widely

dispersed elongated elements in cortical fiber cells, but are aggregated in nuclear fiber cells. Although the size of the filament backbone corresponds to that of actin, positive morphological identification has not yet been made. Nevertheless, it is the likely candidate for the actin molecule that has been found biochemically.

The lens cytoskeleton thus shows regional variations. Microtubules, actin, and intermediate filaments are prevalent in the epithelial cells but chains are lacking. In the mature cortical fiber cells, chains are abundant, whereas microtubules are absent and intermediate filaments are decreased in number. Nuclear fiber cells contain only aggregated chains.

It appears that the interior of the lens fiber cells is occupied by a three-dimensional filamentous network, the interstices of which are occupied by the crystallins. This network, attached to the cell membrane, imparts an internal architecture to the cells that minimizes light scattering, stabilizes the order and distribution of proteins, and maintains the shape of the fiber and the lens.

(5) Intraocular Lenses

There are several grants being supported by the Cataract Program of the NEI in the field of intraocular lenses.

- (a) A randomized controlled trial of intracapsular cataract extraction versus intraocular lens implantation is being conducted by Dr. Hung Cheng. There is a three way randomization between intracapsular extraction, intracapsular extraction with lens implant, and extracapsular extraction with lens implant. Eyes after intracapsular extraction only will be fitted with contact lenses. Vision, complication rate, and patient satisfaction are being assessed.
- (b) Another study, conducted by Dr. R. Smith, is looking at the short and long-term effects of intraocular lenses on the corneal endothelium in patients undergoing cataract extraction. The patients are examined pre-and post-operatively with specular microscopy and corneal pachymetry to determine the endothelial cell counts and the corneal thickness. One hundred patients who underwent extracapsular cataract extraction with and without implantation of a Shearing ocular lens have been enrolled in the study.

This grant was converted from a clinical trial to a natural history study and long term follow up of the 99 patients who have undergone extracapsular cataract extraction with or without a Shearing Intraocular lens.

- (c) A third grant is investigating the mechanisms whereby some patients with intraocular lens (IOL) implants develop protracted uveitis. It was shown by Tuberville et al., that certain components of prosthetic intraocular lenses are capable of activating the

complement of mediating acute inflammatory reactions. Results of the studies indicate that certain nylon-and polypropylene-looped intraocular lenses are not "inert" biologically and may elicit acute inflammatory reactions.

(6) Noninvasive Methods of Lens Research

Noninvasive methods to study the development of cataract are being expanded and refined. Dr. Nai-Teng Yu of the Georgia Institute of Technology has developed a laser Raman spectroscopy technique as an *in situ* structural probe of the ocular lens. He has demonstrated that low power helium-neon laser is a suitable excitation source for detecting the red fluorophor in a brunescent human lens for Raman spectroscopy. The main useful signal that this group is monitoring originates from sulfhydryl groups in proteins and peptides in the eyes. This stretching-mode spectroscopic signal changes during the lifetime of animals and humans. It also changes as cataract forms, so that the development of cataract can be monitored in animal model systems. Those changes seem to occur even before the cataract makes the eye truly opaque. If this apparent correlation holds up to further study, Raman spectroscopy might become a diagnostic method for predicting the development of cataracts, possibly at an early enough stage to halt their progress.

Dr. Jack V. Greiner of the Chicago College of Osteopathic Medicine and Dr. Thomas W. Schleich of the University of California at Santa Cruz are utilizing the techniques of nuclear magnetic resonance (NMR) spectroscopy in lens research. The former group is following the P^{31} NMR spectra of intact rat lenses and rat lens acid extracts. The qualitative and quantitative NMR studies of the intact lens and the lens extract were comparable with respect to levels of phosphorus-containing metabolites of intermediary metabolism. The latter group is using C^{13} NMR studies of carbohydrates on rabbit lenses *in vivo* and *in vitro*. They are able to follow the production of lactate and sorbitol while the lens is being cultured. The development of sensitive probes will enable investigators to make NMR scans of lenses *in vivo* for animals and humans for normal and cataractous states.

(7) Animal Models for Human Senile Cataract

There is always the question as to whether animal models are helpful in human senile cataract research. Recent studies on the Nakano cataract indicate that many changes which occur are quite similar to those found in human cataracts. In Nakano lenses high molecular weight disulfide-linked aggregates, disulfide-linked cytosol polypeptides to fiber membrane, an apparent increase in the concentration of degraded polypeptides, disulfide crosslinking of low molecular weight species, and marked differences in membrane polypeptide profiles were observed. A striking similarity was found between these observations with the Nakano cataract and previous reports of the changes in protein chemistry in the development of senile human cataract. It can be concluded that although the initiating event for induction of the cataract may differ, the sequence of events following such insult may be similar.

The Emory mouse cataract is a late-appearing lens opacity which may serve as an animal model for some human senile cataracts. Many gross morphological and microscopic features resemble findings in senile cataract. As an animal model it has many desirable characteristics. Its slow development permits studies of the lens at the pre-cataractous stage and makes it a good assay system for drugs or other factors affecting cataractogenesis.

In experimental cataracts one consistent finding was that as the cataractous process begins crystallin synthesis ceases. This long standing puzzle has now been unraveled. In the Nakano mouse, Philly mouse, and sugar cataract, changes in electrolytes (increase in sodium and loss in potassium) occur. It has now been shown that the sodium and potassium turn off the translation of messenger RNA resulting in cessation of crystallin synthesis. Messenger RNA is still present, but these electrolyte changes block in some way the reading of the message and consequently protein synthesis.

(8) Tissue Culture

One American investigator has been characterizing the conditions required for the growth of human lens epithelial cells in culture, some of which have been maintained for one year. Under a wide spectrum of culture conditions human lens cells exhibit very limited growth and appear to undergo precocious senescence.

The same group found that insulin growth factor (IGF), the most highly purified of the insulin-like growth factors, was a potent mitogen for mammalian lens epithelial cells; IGF and EGF (epidermal growth factor) triggered cell proliferation throughout the normal amitotic central and preequatorial region of the epithelium. The mitotic response elicited by IGF and EGF was dose-dependent, was preceded by DNA synthesis, and exceeded that engendered by equimolar insulin.

Another investigator has been culturing human lens epithelium-mouse somatic cell hybrids and tumors from these hybrids in order to make gene maps of various human lens functions. This line of study provides information about the biochemical and genetic regulation of lens proteins, specifically basement membrane and crystallins. They are attempting to elucidate the errors that occur in various hereditary and acquired disorder of the lens, such as cataract.

(9) Lens Proteolysis

An investigator is in the process of demonstrating that in the lens, proteolysis is important for normal cell development and function as in other tissues, and that changes in proteolysis occur during cataractogenesis.

A neutral protease, which may be involved in the degradation of the crystallins, has been described to occur in ox lens cortex. However,

neither fractionation to purity nor unraveling of the exact function of the protease has yet been achieved. The neutral protease activity has also been demonstrated in a variety of other species, including monkey, pig, sheep, rabbit, rat, and mouse. Human lens seems to contain only 25% of the amount of protease found in cattle.

Another investigator is approaching the subject in a different way. He has noted the apparent absence of physiologically active proteinases in the normal lens, due to the presence of specific proteins which bind to and inhibit the lens proteinases, thereby protecting the delicate protein matrix of the lens from destruction. He has demonstrated several trypsin-like proteinases and inhibitors in the lens and is purifying them to homogeneity. There seems to be a wide variety of proteinases and inhibitors in the lens and each has a different specificity, mechanism, and chemical stability. Therefore, any disruption of lens chemistry could potentially inactivate one or more of these inhibitors, thereby causing the release of active proteinases. These proteinases in turn can cleave the lens crystallin and promote protein aggregation by permitting the formation of covalent bonds and hydrophobic interactions which are not possible in the normal lens.

(10) Molecular Biology of the Lens

Carper et al., showed that the messenger RNA for a beta-crystallin polypeptide with a molecular size of 27 kilodaltons, first detected 5 to 10 days after birth in the normal mouse lens and the Nakano mouse cataract, was not detected in the Philly mouse cataract with translation in vitro. The heterozygous Philly mouse lens had intermediate levels of the 27 kilodalton beta-crystallin polypeptide and exhibited delayed onset of the cataract. The deficiency of functional 27 kilodalton beta-crystallin messenger RNA is the earliest reported yet for the Philly mouse lens and points to a transcriptional or posttranscriptional developmental defect in the hereditary cataract.

(11) Presbyopia and Accommodation

One of the consequences of aging is the continuous decrease in accommodative amplitude and the development of presbyopia. In all probability the lack of an animal model has resulted in no basic studies to support these conclusions. Bito et al., have initiated a study of the relation of age to loss of accommodative amplitude in Rhesus monkeys in age from 0.5 to beyond 30 years. His group has measured the refractive power and axial dimensions of the eye under resting and fully accommodated conditions. The resting axial thickness of the lens was found to increase with age throughout adulthood, well past the end of the growth period. A strong correlation was found between pharmacologically induced change in the refractive power of the eye and change in lenticular thickness. These similarities to the human condition suggest that the Rhesus monkey represents a highly suitable animal model for the study of accommodation and presbyopia.

(12) Nutrition and Cataract

Very little work has been done in studying the relationship of nutrition and cataract formation in humans. A scattering of studies are being conducted on animal models with respect to nutrition and cataract.

Several investigators are studying the effect of inorganic selenium injected subcutaneously in newly born rats. Shearer et al., are studying the effects of cadmium and selenium on cataract formation. Five groups of suckling rats received daily subcutaneous injections of saline (controls), selenium alone, cadmium alone, or selenium plus cadmium. At approximately 4 and 8 weeks of age, the opacity of the central areas of the nuclear cataracts was scored by slit lamp examination. The results showed: (a) selenium caused very dense nuclear cataracts, (b) cadmium significantly ameliorated cataract induction by selenium, and (c) only minor cataract regression was observed with time with these high doses of selenium.

In another area of study, Podos et al found the parenteral dl-alpha-tocopherol (vitamin E) was effective in arresting 3-amino-triazole-induced cataract in rabbit by about 50%. It also normalized the increased hydrogen peroxide of aqueous and vitreous humor, and malondialdehyde (MDA) of the lens.

Varma has noted that when rat lenses are cultured in a medium containing a photosensitizer such as riboflavin and a fluorescent light source was used, photoperoxidation could be thwarted by the addition to the medium of superoxide dismutase, catalase, or ascorbate (vitamin C). There is thus a suggestion that peroxidative degradation is initiated by photocatalytic generation of superoxide free radical and its subsequent derivation to other potent oxidants. Ascorbate or vitamin C is a strong reducing agent capable of neutralizing some of these oxidants.

Research Needs and Opportunities

Additional studies should be conducted to determine the basis for cataract formation with human aging. Further studies should be made on lens morphology, cell division, and protein synthesis. Increased application of the techniques of molecular biology should be made, including recombinant DNA technology, to the problems of cataract development. Even though the Cooperative Cataract Research Group has initiated a lens classification system combined with photography, further development of an objective, reproducible, and standarized classification of type and severity of cataract needs to be established. Further characterization of light scattering molecules in senile cataract needs to be determined. Further study of the molecular structure of the plasma membrane of lens cells is required. This is where the cataractous process may be initiated, and its interaction with other cellular elements may occur. Further study of

the molecular structure of the plasma membrane of lens cells is required. This is where the cataractous process may be initiated, and its interaction with other cellular elements may occur. Further study of aldose reductase activity is desired to determine in normal and diabetic human lenses whether this enzyme is involved in the refractive changes and fluctuations commonly experienced by diabetics. Investigation should be made of the genetics and natural history of inherited cataracts, including long-term, controlled familial studies. A scientific comparison should be made of various approaches to cataract surgery. Toxicological studies of intraocular lenses should be made. Studies are required to determine the mechanisms of inflammation induced by intraocular lenses with the aim of developing improved devices. An evaluation should be made of proposed medical (nonsurgical) methods of cataract treatment.

IV. GLAUCOMA
FISCAL YEAR 1982

Introduction:

Glaucoma is a disease characterized by loss of peripheral vision due to irreversible damage to the optic nerve. It is generally characterized by abnormally high intraocular pressure, changes in the appearance of the optic nervehead, and a pattern of losses in the visual field. In one type of glaucoma, "low tension" glaucoma, intraocular pressure is within normal limits. In many people high intraocular pressure, ocular hypertension, is not accompanied by signs of nerve damage or loss of vision. It is not known what causes the susceptibility of an individual optic nerve to damage at a given level of intraocular pressure. Early detection and treatment can arrest or slow progress of the disease, but once vision is lost it cannot be restored.

Glaucoma is primarily a disease of the aging, although it may occur at any time in life. It is one of the major causes of blindness in the United States. Over 62,000 Americans are blind from glaucoma, over 200,000 have severely impaired vision, about 1.5 million suffer the disease, and an equal number may be unaware of their disease. Estimates suggest that as many as 1/3 of the people newly diagnosed as having glaucoma present with low tension glaucoma, in some of them pressure becomes elevated with time. As many as 10 million people may have ocular hypertension. A significant number of ocular hypertensives will ultimately develop glaucoma, but there is no way at present to detect which individuals are at risk and should be treated. At present, glaucoma is treated by controlling the level of intraocular pressure by drugs or by surgery. Normal intraocular pressure is maintained by balancing the rate of production of aqueous humor by ocular tissues with the rate at which it leaves the eye. Fluid is formed by ciliary tissues, flows forward between the iris and lens into the anterior chamber, and leaves the eye by filtering through the trabecular meshwork, which occupies the angle formed by the iris and cornea, and drains through the canal of Schlemm. Almost always, it is impeded filtration, rather than overproduction of aqueous humor, which causes an elevation of intraocular pressure. Drugs which control glaucoma may act to diminish aqueous humor production or to increase its outflow from the eye, and the most commonly used surgical procedures improve outflow.

There are several types of glaucoma. Primary open-angle glaucoma (which accounts for up to 80 percent of all cases), low tension glaucoma, and ocular hypertension are characterized by anatomically open angles - there is no apparent anatomic reason for impeded aqueous humor outflow. Angle-closure glaucoma represents 10-20% of the glaucoma cases. In this condition a narrower than normal filtration angle is caused by a more anterior positioning of the iris and lens, and if lens and iris come into apposition and are pushed forward, the angle is blocked. Surgical creation of a hole, in the iris, iridectomy, relieves angle-closure glaucoma. Glaucoma in infants and children results from congenital or developmental anatomic defects in the anterior chamber. The underlying causes for the variations of the disease are poorly understood and poorly classified and improved methods of treatment are required. While some types of infantile or childhood glaucoma can be treated successfully, others may culminate in

life-long visual impairment or blindness. Secondary glaucomas result from a number of systemic and ocular diseases. Some of these kinds of glaucoma are very difficult to treat and lead to an incidence of blindness much higher than that due to the much more prevalent open-angle glaucoma. The most severe of these diseases are neovascular and inflammatory glaucoma.

The research objectives for the glaucoma program have been set for the next five years by the National Advisory Eye Council, in Vision Research: A National Plan 1983-1987. Major program goals and an outline of the research objectives for glaucoma are set forth below.

Program Goals

- o To conduct clinical and laboratory studies to define genetic, racial, and environmental risk factors for the development of damage to the optic nerve in glaucoma and to determine the reasons for the varying susceptibilities of individual eyes to optic nerve damage.
- o To develop improved methods of detection and diagnosis and better drug and surgical treatments for glaucoma.
- o To devise new noninvasive clinical techniques for studying aqueous humor formation and flow, for continuous monitoring of intraocular pressure in man and in primates, and for studying experimental optic nerve damage in monkeys.
- o To study the cell biology and molecular characteristics of the outflow tissues and their abnormalities in glaucoma, especially using tissue and organ culture techniques.
- o To exploit experimental methods of studying the basic physiology and pharmacology of fluid movement in the eye and the control of aqueous humor inflow in primates and in man.
- o To investigate neovascular glaucoma which results from new abnormal blood vessels on the iris overgrowing the angle, often occurring as a severe complication of certain retinal vascular diseases, and to develop methods for its treatment.
- o To establish eye donor programs so that appropriately obtained and preserved human tissues from well-characterized glaucoma patients will become available for correlations between clinical history and tissue damage and for cell biology studies.

Program Structure

Research on glaucoma is classified by subcategories of the disease. Because open-angle glaucoma is the predominant type of glaucoma, elements common to all types of the disease are considered under this heading.

A. Primary Open Angle Glaucoma

1. Etiology, Epidemiology, Management, and Therapy
2. Aqueous Humor: Inflow
3. Aqueous Humor: Outflow
4. The Optic Nerve

B. Angle-Closure Glaucoma

C. Developmental, Congenital, or Infantile Glaucoma

D. Secondary Glaucomas

Recent Accomplishments

Marihuana Update

The possible benefits of cannabinoids in the treatment of glaucoma remain a subject of considerable public and scientific interest. While there is documentation that smoked marihuana or delta-9-tetrahydrocannabinol (THC) reduce intraocular pressure (IOP) in some normal individuals and some glaucoma patients, there is no scientific evidence that THC would be a safe and effective drug for the chronic treatment of glaucoma. In addition to its well known effects on behavior, including impaired motor performance, inhaled marihuana or THC smoke produces variable effects on cardiovascular performance, including transient increases in blood pressure or heart rate or, more dramatically, a sharp drop in blood pressure. Occasionally, transient increases in IOP have been observed.

Since virtually nothing is known about the ocular pharmacology of cannabinoids, and because intraocular pressure can be controlled for 85-95% of open-angle patients by use of existing treatments, it seems premature to suggest cannabinoids as therapeutic agents at this time. For example, information is lacking in all areas which must be understood before any drug could be used; we especially need to know the following about marihuana:

- o Is marihuana safe to use in aging glaucoma patients? Little is known about its reactions in the elderly, and orthostatic or postural hypotension (fainting) has been observed in smoking tests with marihuana or THC.

- o Is marihuana safe to use in multiple daily doses over a prolonged period of time to keep IOP down? What are its chronic systemic or ocular side effects?
- o Is it effective following prolonged use, or as with many drugs, does its therapeutic effect diminish with time, or are increased doses required to maintain a therapeutic effect?
- o Does it act upon the eye in ways different from the actions of drugs currently in use, e.g., would it complement or replace any of the drugs commonly used? Until its mechanism of action is understood and it can be demonstrated that a potential anti-glaucoma drug offers unique benefits, it is not reasonable to consider introducing it into clinical use.
- o Are there safer and more effective modes of administration of marihuana, its derivatives, or analogs which would lower IOP but eliminate its undesirable physical and behavioral side effects?
- o Most importantly, does marihuana's mechanism of action on the eye in lowering intraocular pressure have any adverse effect on other ocular function? For example, some potential ocular hypotensive agents were found to diminish the blood supply to the optic nerve and actually worsen the effects of glaucoma.

Active NEI-sponsored research is aimed at answering several of these questions. Dr. Coy Waller has found that cannabinoids other than THC have some ocular hypotensive effects in rabbits and monkeys, and their semi-synthetic derivatives or synthetic analogues may have drug potential. New studies show that THC affects an ocular enzyme system hitherto not implicated in control of aqueous humor hydrodynamics, monoamine oxidase; this finding may be of importance in explaining the mechanism of drug action.

Characterization of a non-cannabinoid aqueous extract of marihuana with potent IOP lowering activity by Zalkow, Green, et al. is progressing; this promising material which would not carry the cannabinoid stigma, may be free of the undesirable physical and behavioral effects of marihuana. The partially purified substance is extremely potent in rabbits, as 5 ug injected intravenously to an animal reduced IOP by as much 60% in 7-8 hours. However, in its present form, it has no effect on topical administration, and no hypotensive effect can be elicited in monkeys. Chemical, biochemical, and pharmacologic means are being used to purify and characterize this glycoprotein material, and indeed, species specificity may reside in its carbohydrate moieties. It is not an adrenergic agonist, nor does it interact with receptors for other major neurotransmitter systems, but its effects can be blocked by sugars. Thus it seems to be a unique compound which acts upon hitherto undescribed receptors of the ciliary body to drastically reduce aqueous humor formation. Its immediate potential is as a research tool which may aid in our understanding of the production of aqueous humor; ultimately, the pure compound or a derivative of it may have clinical application.

Laser Iridotomy

An estimated 30,000 argon laser iridotomies are now performed annually in the United States to relieve angle-closure glaucoma, either therapeutically in affected eyes or prophylactically in fellow-eyes of angle-closure patients. In the last few years, this procedure has largely replaced the very successful surgical treatment, iridectomy. Pollack and Robin and Quigley have reported on series of 140 and 98 laser iridotomies respectively, in all of which satisfactorily draining holes were produced in the iris. At 1-5 years after treatment, in most patients visual acuity was preserved as well as by iridotomy; in the others, cataract progression (at a rate comparable to that of aged people) was responsible for the lessened visual acuity. The two surgical procedures controlled IOP in about the same way with respect both to needs for post-treatment medication and for ultimate filtering surgery.

A large scale clinical trial to compare iridectomy and laser iridotomy has not been mounted, as accumulating clinical experience has convinced surgeons that most patients are served at least as well by the laser procedure and are spared the pain, costs, and inconveniences attending hospitalization and surgery. One group of investigators attempted to conduct a randomized clinical trial (performing an iridotomy in one eye and an iridectomy in the other of each patient). Ten patients were entered into the study and underwent both procedures. No differences in subsequent visual function were apparent and both procedures were judged to be equally satisfactory in outcome. It became increasingly difficult to recruit patients as they were either referred for iridotomy or insisted upon it. The ophthalmologists meanwhile are following a series of about 100 iridotomy patients, who could or would not be treated surgically. No significant problems have been observed and no losses in visual acuity were observed. The randomization protocol was dropped because the investigators felt it would be unethical to continue offering the surgical procedure with its obvious disadvantages relative to iridotomy.

In experimental studies, sequential histologic studies of primate eyes subjected to laser iridotomies showed that particulate matter was released from the iris and that it accumulated in the angle, that pigment was seen first in the juxtacanicular meshwork, and then both extra- and intracellularly in endothelium and Schlemm's canal. These observations suggest that pigment leaves the anterior chamber by combined bulk flow and phagocytosis. No increases in IOP were observed post-operatively, and at one year after iridotomy, no persisting damage to angle tissue was observed.

We have estimated that the aggregated national benefits of the switch from iridectomy to the out-patient argon laser treatment amount to about \$66 million per year. Lost productivity is diminished from an average of 10 days to 0.5 day, hospital fees averaging nearly \$1000 are eliminated, and surgeon's fees are reduced, leading to a conservatively estimated savings of \$2,200 for each of 30,000 patients.

Laser Trabeculoplasty

A series of about 80-100 argon laser burns spaced circumferentially on the trabecular meshwork lowers intraocular pressure in open-angle glaucoma patients, at least in the short-term. This brief out-patient procedure produces only minor transient irritative side effects. It appears to act by shrinking the scarred areas and thereby exerting tension on the meshwork which lifts it away from the canal of Schlemm to improve aqueous humor outflow. Evidence supporting this mechanism was presented at the 1982 meeting of ANGLE, the glaucoma research group. Two investigators had used the procedure on enucleated eyes or excised trabecular rings and observed shrinkage. Also, scanning electron microscopy and histologic methods were employed to follow tissue changes. SEM of laser-treated angle tissue clearly showed the canal of Schlemm beneath an uplifted coagulated layer of trabecular tissue.

Laser trabeculoplasty is being increasingly employed as a substitute for filtering surgery or in patients unable to tolerate maximum medical therapy. While it seems to be a safe and effective procedure, occasional side-effects have been noted; however, it has not been in use long enough to allow for evaluation of long-term safety and efficacy. As with most new procedures, initial anecdotal reports are almost uniformly favorable. Given the enthusiastic reception of laser trabeculoplasty, its apparent benefits relative to risks, and its widespread use, there is some concern that if a controlled, randomized clinical trial does not define both benefits and risks, it will come into premature use, possibly in inappropriate categories of patients.

It is anticipated that a long-term clinical trial will be initiated in FY 1983, to investigate some important aspects of laser trabeculoplasty. The investigators of the projected study anticipate that there will be increasing pressure to offer this treatment as an alternative to medical therapy for glaucoma and hope to forestall such an expansion until many important questions can be answered under the conditions of a specifically designed randomized study. In this trial, newly diagnosed open-angle glaucoma eyes will be randomized to either laser trabeculoplasty or conventional therapy with each patient being his own control. Eyes will be followed longitudinally, and needs for changes in medicine, further laser treatment, or surgery will be noted. Also, IOP, visual acuity, optic nervehead appearance and visual fields will be monitored.

Estimates can be made, knowing the incidence of open-angle glaucoma, hospital costs, the social costs of lost productivity and blindness, for the savings that could ensue if laser trabeculoplasty were indeed to substitute for most medical and surgical treatments of open-angle glaucoma. If 90% of patients eligible annually for surgery could be treated with the laser, an estimated annual savings of \$55.2 million might be realized (the costs of treating about 24,000 patients, estimated at \$2300 each for surgeon's fees, hospital costs, and lost productivity). Indirect savings, occasioned by reducing by 20% the annual rate of onset of blindness, the attendant the need for nursing home care, social security costs, and by increasing the productivity of 15% of those in whom blindness might be avoided, could total as much as \$250 million per year. If the need for

anti-glaucoma drugs could be halved, almost \$40 million would be saved annually. Thus, if much of the medical and surgical treatment of glaucoma could be avoided by treatment with laser trabeculoplasty, the nation's social and economic benefits would be sizeable.

Outflow Physiology and Cell Biology

Barany, Kaufman, and associates have been able to differentiate drug effects on the aqueous humor outflow system into those acting upon the iris muscle, the ciliary muscle, or the trabecular meshwork. Drugs like epinephrine or norepinephrine act upon both inflow and outflow mechanisms and upon both alpha- and beta-adrenergic receptors. These multiple actions have confounded efforts to define their pharmacophysiologic properties. The alpha-adrenergic actions on the iris and ciliary muscle have been thought to increase outflow by miosis which exerts traction on the trabecular meshwork. Aqueous humor physiology was studied in cynomolgus monkeys with total iridectomies, disinserted ciliary muscles, or having had both surgical procedures. Perfusion studies using drug dosages comparable to those in clinical use showed that outflow facility was increased roughly equally in operated and control eyes, ruling out alpha-adrenogenic effects on iris muscle and indicating that epinephrine and norepinephrine cause an increase in outflow facility by direct action on the trabecular meshwork.

Another approach to understanding the control of aqueous humor outflow and its impairment in glaucoma is to measure properties of the cells in the outflow pathways. Tripathi has studied the contractile proteins of the trabecular cells by electron microscopy and by using fluorescent antibodies. Electron microscopy revealed that less actin was present in the glaucomatous eyes than in the controls, and actin-specific immuno-fluorescent reactions of tissues from eyes with glaucoma were markedly less than those of normal eyes.

Presently, it is not known if the reduction of actin in trabecular endothelial cells of eyes with glaucoma is primarily related to the disease process or is secondary to the use of anti-glaucoma drugs. The density of trabecular meshwork cells decreases with age. This was shown in a painstaking quantitative microscopic study by Alvarado et al. of specimens of eyes obtained from normal humans aged from birth to 81 years. A regression line of cellularity versus age was plotted and compared to similar data obtained from glaucomatous eyes; the lines were parallel, but significantly displaced, suggesting that glaucoma patients have lower trabecular cell densities at all ages than control individuals, and that this loss of cellularity may precede their overt disease.

Characterization of the properties of cultured trabecular endothelial cells is a major ongoing effort in the laboratories of Polansky, Palmberg, and Southern. Trabecular cells exhibited high affinity binding for ligands for a beta-adrenergic reagent and essentially no binding of an alpha-adrenergic probe. Also, glucocorticoids suppressed thymidine uptake by the cultured endothelial cells, quite in contrast to their effect on cultured skin fibroblasts; this observation finally makes the point that the ocular targets for glucocorticoids cannot be inferred from their effects on peripheral cells. Glucocorticoids bind specifically to cultured human

trabecular meshwork cells with an affinity of 5 nm and about 60,000 receptor sites per cell. The continuing studies of Southern et al. have established that in rabbit trabecular tissues, as in the rest of the body, cytoplasmic receptors bind topically applied glucocorticoids in a dose and time-dependant manner and translocate them to the nucleus.

Southern et al. cultured outflow pathway tissues in the presence of precursors of extracellular matrix materials, the glycosaminoglycans (GAGs) and collagen, and measured the effects of dexamethasone on their biosynthesis. This is a significant study because it may be that diminished aqueous humor outflow is neither always nor necessarily determined by collapse or contraction of the trabecular structure; rather, outflow may be diminished by changes in the nature or density of the extracellular macromolecular structures through which aqueous must percolate. Steroid significantly decreased the incorporation of GAGs into outflow cells and increased collagen precursor uptake (no change was noted in general protein precursor uptake). Knepper had shown earlier that GAG content of rabbit anterior segment tissues changed significantly following topical instillation of dexamethasone. Recently, using microdissected trabecular meshwork and adjacent tissues, he has isolated, characterized, and quantitated GAGs. These findings extended to human and primate cells may determine if changes in extracellular matrix diminish outflow in glaucoma and if extracellular materials are changed by drug treatment.

Of possible future clinical interest are the reports of Bito et al. that at low dosages prostaglandins PGE_2 or PGF_2 reduce IOP in cat and rhesus monkey eyes. Since these agents have been implicated in ocular inflammatory responses and disruption of the blood ocular barrier, they have generally been considered deleterious to the eye. At the very low doses employed in this study such effects were not noted, nor were the early ocular hypertensive effects of the prostaglandins seen. PGE_2 instilled in monkey eyes produced a significant reduction of IOP and no miotic response, while PGF_2 reduced IOP and produced profound miosis. That low doses of PGE_2 reduces IOP in rabbits also has been observed by Moses, in using perfusion experiments with eyes obtained at specific times after topical drug instillation. IOP was lowered by a single dose of PGE_2 by an increase in true facility of outflow lasting for up to six hours.

Optic Nerve Studies

One of the most important research objectives in glaucoma is the development of new noninvasive means of detecting signs of the earliest disease changes in the optic nerve. Zeimer has undertaken to measure optic nervehead rigidity (stress-strain relationships at the lamina cribrosa) by use of an instrument he is developing, a laser doppler velocimeter. It is hoped that such an instrument will be of aid in determining which ocular hypertensives are developing changes at the optic nervehead and need treatment to lower IOP. An instrument has been built and tested on animals that shows a favorable signal-noise ratio, and at this stage, reproducibility of 15 percent in measurements of optic nervehead displacements of 50-100 microns. The instrument has been devised so that blood flow and eye movements will not

interfere with the measurement. The laser energy employed is well below the limit approved for retinal exposure. If continued development and testing on primate and human eyes are successful, this instrument could have important clinical applications.

Hart is continuing to analyze longitudinally patterns of field changes in open-angle glaucoma patients. Of 63 eyes having glaucomatous defects and under continuous medical treatment and showing only marginally elevated levels of intraocular pressure for ten years, 73% progressed to a dense involvement of the affected field area. Kinetic Goldmann perimetry was used routinely; initial scotomas were confirmed by meridional threshold static perimetry and most recently by this investigator's computerized method of displaying three dimensional contours of the field. Earliest defects were shallow scotomas, which in some eyes were transient or intermittent, and later defects appear to have involved the same nerve fiber bundles. Later field defects were denser than the first, but their appearance could not be correlated with variations in IOP. Most of the involved eyes showed a very gradual rate of increase in the defects; a minority showed a relatively abrupt onset and rapid progression of the defect. Nerve damage in glaucoma appears to fall into three phases over roughly 10 years: occult damage with no field defect detectable even though IOP is high; a threshold period of transient, barely detectable shallow defects; and finally, a critical period in which defects become dense, and in which remaining nerve fiber sensitivity is high (IOP may be only marginally elevated). It is discouraging that, in closely monitored glaucoma patients under continuous care and having "controlled" IOP, the disease, once established, continues to progress. These observations show the need for improved methods for very early detection of glaucoma and better methods of treatment and once again indicate the poor correlation, for a given patient, between "normal" IOP and the susceptibility of the optic nerve to damage.

Quigley et al. examined 23 glaucomatous eyes obtained at enucleation and correlated the histologic features of their optic nerve fibers with prior clinical measurements. A loss of axons was found to precede reproducible visual field defects in some glaucoma suspects. Nerve losses were greatest at the superior and inferior poles, a pattern which correlates with anatomic differences in the distribution in the lamina cribrosa of pore sizes in and thinness of the connective tissue sheaths through which the fibers pass. This suggests that pressure-induced physical distortions in that region produce nerve damage. However, other evidence suggests that pressure-induced ischemia destroys nerve fibers.

These findings emphasize the importance of experimental studies of the basis of optic nerve susceptibility to damage in glaucoma. Four groups of NEI-supported investigators are studying this problem using primate eyes. Radius and Anderson have used axonal transport and electron microscopic studies to describe the effects of acute elevation of IOP on the optic nerve. Previously, they had shown that, at moderately high perfusion pressures, interrupted axonal transport was reversible if pressure were reduced to normal within 8 hours. Recently, more extreme pressure effects

were examined. One-half to 2 hours of high IOP produced hydropic swelling and necrosis in the anterior lamina cribrosa, the optic nervehead, and the retina, and retrograde fast axonal flow was interrupted in the posterior third of the lamina cribrosa. For pressure insults of under 4 hours damage was partially reversible. Eyes studied by electron microscopy 2 weeks after pressure insult showed that 4 hours increased pressure caused diffuse axon atrophy across the optic nerve, while partial atrophy followed shorter periods of increased pressure. Pressures less than 10 mm above ophthalmic artery pressure caused little or no irreversible damage to the nerve. Thus, the extent of optic nerve damage due to acute high IOP is limited to the posterior lamina cribrosa, and the reversibility of axonal damage indicates that axons tolerate ischemia better than cell bodies, e.g., retinal ganglion cells. Moreover, pressure-induced blockade of axonal transport is reversible, leaving still open the question of whether irreversible pressure-induced optic nerve damage is due to ischemia or mechanical factors.

In another study, these investigators studied electron micrographs of the lamina cribrosa of owl monkey eyes made after four hours of elevated IOP. Cross-sections of the optic nerve indicated that damaged axons in either the periphery or the center of a bundle were equally liable to damage, and that within a bundle, damaged axons were randomly dispersed. This pattern seems to rule out both ischemia and pressure-induced kinking at the lamina as a cause for interrupting transport. Anderson and Sossi have measured blood flow in the posterior segment of cat eyes at elevated levels of IOP. At IOP levels sufficient to reduce blood flow in the retina, choroid, and optic nervehead, the flow in lamina cribrosa and intraorbital optic nerve was not affected. Only at an extreme pressure elevation was the lamina's flow affected. Thus, it appears that there is an efficient autoregulation of blood flow in the optic nerve, and unless that process is compromised in glaucoma, ischemia per se does not cause glaucomatous cupping. Recent evidence favors elements of both the ischemic and mechanical theories of the mechanism of optic nerve damage by high IOP, but the question is still unresolved.

Development of The Anterior Chamber and Its Defects

Anderson has reported on his observations, by light, scanning, and electron microscopy, of angle tissues obtained from developing eyes and a series of eyes with infantile glaucoma obtained at various ages. Observations during normal development, show that the inner surface of the trabecular meshwork (TM) is exposed to the anterior chamber after 20 weeks as the ciliary muscle and processes recede behind the scleral spur. As the TM is exposed, it does not have an endothelial covering; rather it is seen as the surface of a multi-layered mass destined to become the TM endothelium. By seven months, cells at all levels separate and the trabecular tissue cavitates, and the extracellular matrix develops into sheets of endothelium and beams. This process distinctly is not viewed as disintegration of an endothelial layer. Meanwhile, the endothelial lined and vacuolated canal of Schlemm has developed and apparently accommodates aqueous humor flow. The three dimensional nature of the TM is apparent during development.

In infantile glaucoma, the iris and anterior ciliary body appear not to have moved posteriorly as far as in normal eyes, being at the position seen at 7 to 8 months in the fetus, and they overlap the posterior TM. Also, the beams are thicker than normal. No covering membrane was seen on any specimens. However, on a specimen retrieved for electron microscopy from paraffin, the TM did appear as compressed sheets, separation of which might have given the appearance of a membrane, and this may explain some classic descriptions of a membrane. Schlemm's canal is normal, but without vacuoles. In no event does the author believe that goniotomy slices into an imperforate membrane; rather he indicates that when the innermost tightly packed TM sheet is cut, traction on the iris is released. He postulates that the primary developmental defect in infantile glaucoma is abnormally thickened sheets of TM cells and/or excessive formation of collagen beams. The thickened sheets may prevent the normal posterior movement of the uveal tract, resulting in the observed anterior displacement of iris and ciliary body. Another possibility is that trabecular sheets are compressed one against another, and that incision relieves the compression; this would account for the success of goniotomy or trabeculectomy in treating infantile glaucoma. Kupfer and Kupfer-Kaiser have hypothesized that anterior chamber developmental anomalies result from abnormalities in migration or terminal induction of the neural crest cells which differentiate into anterior ocular tissues.

Townes-Anderson and Raviola have continued to describe the morphology of the anterior segment in the developing rhesus monkey eye. In one study, prenatal vasculature was examined by all levels of microscopy, and vessel permeability was examined by following intravascular tracers. Unlike most of the prenatal vessels, the vasculature of the developing uveal tract is highly permeable to horseradish peroxidase (HRP). These vessels are fenestrated and their endothelial cells are joined by open junctions. These uveal vessels penetrate the developing ocular chambers, then as the anterior chamber matures they recede into the iris. HRP diffuses into the ocular fluids from these vessels and from the uveal mesenchyme at the angle, a process which stops just before birth. These observations can explain the observed presence of plasma components in the immature eye, and the changing composition of the aqueous during development.

Areas of Developing Interest

The roles of several autacoids, neurotransmitters and neuromodulatory substances, and mediators of the inflammatory response, are being elucidated rapidly in the central nervous system, stimulating investigations into whether they are present in ocular tissues and what roles they may play in normal ocular tissue physiology or in ocular pathophysiology. Mandahl and Bill studied the role of nerve conduction in acute experimental uveitis induced by antidromic trigeminal nerve stimulation and biologically active substances. Use of specific inhibitors allowed them to isolate specific effects. Trigeminal nerve-stimulated IOP increase and miosis were blocked by tetrodotoxin (TTX) which stops nerve conduction. TTX also blocked the IOP rise caused by prostaglandins, but this was overcome by larger PGE doses. Substance P - induced miosis was not affected by TTX, but its elevation of IOP was delayed. Thus, nerve conduction is involved in the rises in IOP caused by PGE and SP, probably via an axon reflex in the anterior uvea causing transmitter release in the ciliary processes. Nerve conduction, accompanied by release of SP, seems to be necessary for the miotic effects of PGEs, while SP can induce miosis without nerve conduction.

The irritative response of the rabbit eye includes miosis, a rise in IOP, vasodilation, breakdown of the blood aqueous barrier. One aspect of this reaction involves prostaglandin release and neurogenically stimulated release of substance P at sensory nerve endings. Inflammatory responses can be elicited by PGs and reduced by use of specific PG inhibitors. Recently SP-immunoreactive fibers have been demonstrated in the iris-ciliary body (quantities diminished by denervation), gasserian ganglion, and cornea of the rabbit. Sears et al. and Cohen et al. showed that SP produced a dose-related strength of contraction of isolated bovine pupillary sphincter which was not affected by antagonists to adrenergic or cholinergic drugs, histamine, prostaglandins, or nerve conduction, indicating a reaction with specific SP receptors. Since substance P is known to be released by afferent pain fibers, it may be that stimulation of the ophthalmic branch of the trigeminal nerve produces pain and triggers an oculopupillary reflex which dilates iris capillaries and constricts the pupillary sphincter. Thus the signs of iritis, swelling and miosis, may be due to sensory fiber stimulation and SP release. Unger et al. point out that the ocular irritative response is mediated by peripheral sensory elements and that the stimulus is propagated and augmented by axonal reflex. This implies that the response to ocular injury is activation of the fifth nerve elements as a final common pathway. Immunoassay of possibly implicated neuropeptides of ocular tissues from normal and denervated rabbit eyes showed that highly significant reductions in substance P content occurred in conjunctiva, cornea optic nerve, and iris-ciliary and choroid in denervated eyes.

Future Needs

A program should be established to obtain and distribute appropriately preserved tissues from eyes of patients at various stages of glaucoma so that we may better understand the disease. An increased level of effort should be devoted to early detection of low tension glaucoma, to determining its etiology, and to improving methods of treatment. Epidemiologic studies are needed to determine why patients with glaucoma lose vision, (e.g., the roles of time of entry into therapy, compliance with therapy, iatric factors) and the roles of genetic or environmental factors in the onset and progress of the disease.

Most drugs now used to treat glaucoma primarily diminish inflow of aqueous humor, and increased efforts should be made to improve outflow with drugs. Better understanding of the physiopharmacology of drug action is needed, and information on how drugs interact with the tissues to relieve elevated intraocular pressure should be sought by *in vitro* studies using organ and tissue culture. The availability of cultured trabecular meshwork cells now makes it possible to study directly the cell biology and drug interactions of the outflow tissues and to determine the basis for resistance to outflow. Many aspects of laser trabeculoplasty must be critically evaluated as it is being substituted for conventional surgery, and to forestall its premature use as a substitute for drug therapy. Many parameters of laser treatment will require careful clinical studies, including clinical trials to determine optimum use of this tool.

The basis of nerve damage in glaucoma is still poorly understood, as is the nature of the correlation between levels of intraocular pressure and extent of nerve fiber loss. The major needs in optic nerve research are for better ways to evaluate the status of the optic nerve in glaucoma and to predict which eyes at risk require aggressive treatment to lower intraocular pressure. The time and technology are now ripe for development of new types of noninvasive measurements for evaluation of parameters of optic nerve health and function. Appropriate therapy and effective preventative measures ultimately will depend upon a thorough understanding of the pathogenesis of optic nerve damage. Efforts should be devoted to experimental studies of glaucomatous optic nerve pathology primarily in primates.

A critical need in forestalling damage due to angle-closure glaucoma is to be able to predict which eyes are liable to suffer closure so that prophylactic measures may be taken. The pathogenetic mechanisms of angle-closure remain to be fully defined.

Research on developmental, clinical, and infantile glaucoma has been hampered both by their low incidence and the lack of common definitions which hinders analysis of data obtained by various investigators. Definitive studies are required to determine the prevalence, natural history of these diseases, and the best means of treating them. Better means for evaluation of aqueous humor dynamics in children are needed. Cooperating regional centers should be established to conduct research into understanding and treating glaucoma in infants and children.

Secondary glaucomas, which result from a number of systemic and ocular diseases, are very difficult to manage and lead to a disproportionate incidence of blindness. The most severe forms of secondary disease are neovascular glaucoma and glaucoma following ocular inflammations.

Some secondary glaucomas are quite rare, which impedes improving our understanding of them. The major needs in the understanding of the secondary glaucomas are for increased research on the pathogenesis and management of neovascular glaucoma and on the genesis and treatment of glaucoma secondary to inflammation. Some of the low frequency types of glaucoma are poorly understood and therapeutic methods need to be compared and evaluated. Cooperative studies are needed to assure that sufficient numbers of patients with these diseases may be seen and their diseases studied thoroughly.

V. STRABISMUS, AMBLYOPIA, AND VISUAL PROCESSING
FISCAL YEAR 1982

Introduction:

Seeing involves a series of highly complex events that begin the instant images fall onto the retina and continue until objects are perceived in all their details, depth, and color. Visual processing is always accompanied by searching and scanning eye movements and is further refined by converging and focusing the eyes onto objects. A disturbance of any one of the many parts of this elaborate and precise system can lead to serious visual disturbances, such as amblyopia, visual field defects, strabismus, nystagmus, and myopia. Because these conditions affect more than 10 percent of the population, they constitute serious public health problems.

The National Eye Institute's Strabismus, Amblyopia, and Visual Processing program supports research on the structure, function, and development of the extraocular muscles and those portions of the brain that make vision possible. Such research is directed toward gaining a better understanding of normal vision and the causes of visual deficits and blindness that do not appear to result from specific dysfunction of the eye itself. This program is committed to support research aimed at preventing or treating strabismus (commonly known as cross-eye or walleye), amblyopia (commonly known as "lazy eye"), myopia (nearsightedness), and neuro-ophthalmological disorders. Understanding visual processing and its disorders requires a working knowledge of the human nervous system and related molecular, genetic, chemical, cellular, and integrative neural processes, as well as overt perceptual responses. Continued advancement of clinical investigation rests upon an improved understanding of basic visual mechanisms; thus, both basic and clinical research are necessary for the development of new methods for diagnosing and treating visual disorders. Although disorders of visual processing may not always cause total blindness, they nonetheless may seriously diminish the quality of human life.

Program Goals:

- o To understand the mechanisms controlling the development of the central visual system, including its modifiability by endogenous and exogenous factors.
- o To develop clinically useful, noninvasive methods of assessing visual capacities in adults and, especially, infants and young children.
- o To define at molecular, cellular and systems, and behavioral levels the normal and abnormal processing of visual information.
- o To use this knowledge to devise better strategies for preventing and treating amblyopia and other neurosensory disorders.

- o To understand the development, structure, and function of the neural and muscular systems that control eye movements, including the variety of subsystems involved in fixating and tracking objects and the interaction of the visual and vestibular sensory systems.
- o To understand the accommodative process and its relationship to vergence eye movements, especially during infancy and in early childhood disorders of ocular motility.
- o To devise better surgical, pharmacological, and behavioral strategies for managing strabismus and other neuro-ophthalmological disorders of ocular motility.
- o To determine the etiology and course of development of myopia and other refractive errors in order to prevent their occurrence or progression.

Program Structure:

In order to reach the overall goals established for the program, research is supported within the following categories:

Subprogram/Areas:

- A. Visual Processing and Amblyopia
 - 1. Normal and Abnormal Development
 - a. Molecular
 - b. Cell and Systems
 - c. Behavior
 - 2. Structure and Function
 - a. Molecular
 - b. Cell and Systems
 - c. Behavior
 - 3. Disorders
 - a. Amblyopia
 - b. Sensory Neuro-Ophthalmic Disorders
- B. Ocular Motility and Strabismus
 - 1. Normal and Abnormal Development
 - 2. Structure and Function
 - a. Conjugate Eye Movements
 - b. Vergence and Accommodation
 - c. Muscle Structure and Physiology

3. Disorders
a. Strabismus
b. Motor Neuro-Ophthalmic Disorders

C. Optics and Refractive Errors Including Myopia
1. Optics and Refractive Errors, Including Myopia

D. Visual Impairment and Its Rehabilitation
1. Low Vision
2. Blindness

Recent Research Accomplishments:

Transplantation of retinal and neural tissue into the midbrain

Preliminary studies have already shown that retinal and brain tissue can be transplanted to other animals under certain conditions. Research supported by grants from the NEI is aimed at determining how best to perform the transplants, how the transplants develop in the host animals, and whether such transplants can achieve normal function.

Tissue sections or reaggregated cells from portions of the visual system of embryonic rats have been transplanted into the midbrain region of newborn and adult rats by McLoon and Lund at the Medical University of South Carolina. Such transplantation has involved tissues from the retina, the superior colliculus, and the cortex. These transplants can survive, differentiate, and form specific connections to appropriate cells in the brain of the host animal and appear normal in many respects.

While connections from the transplant to the host brain seem to form reasonably easily and specifically, formation of the appropriate connections from the host to the transplanted tissue has been more problematic: Although transplanted superior colliculus tissue does appear to be driven by appropriate cell connections, there is little or no evidence of inputs from the host nervous system to transplants of retinal or cortical tissue. The transplantation works best when fetal tissue is transplanted to the brains of newborn rats. Transplantations of fetal tissue to the brains of adult rats also works, but less well. The techniques of transplantation are making it possible to study the development of components of the visual sensory system, how innervation patterns are formed, and the effects of specific lesions to the retinal transplants.

Cell division in the transplanted tissue proceeds for a while and then stops on a schedule that appears to depend mainly on its normal embryonic age and not on when the transplantation occurs. However, fetal retinal tissue can be held in tissue culture up to two weeks prior to transplantation to the superior colliculus of newborn host rats. Retinal cells from the embryonic rats can be dissociated into individual cells, reaggregated, then transplanted to host brains; they still develop connections just as if the transplantation occurred directly after removal from the fetal rat. The investigators have had little or no problem with rejection of the transplanted tissue, even when transplantation is performed between outbred strains of rats. It seems likely that this is due to the existence of the blood-brain barrier, which would limit accessibility of the immune system to the brain.

These preliminary studies promise to tell us much about normal development of various components of the visual processing system. Furthermore, they offer the promise that, someday in the future, human retinal transplants can help blind people to acquire some form of visual sensitivity, and that certain forms of brain damage can be repaired.

Laminar distribution of neural peptides in the optic tectum:

In recent years, a large number of peptides and other small molecules have been discovered in the brain. Some of these substances clearly function as neurotransmitters and some function in the development and maintenance of proper neuronal connections in the brain. Identification and localization of these substances are revealing complexities of the brain that previously had not been identified, and these studies are providing considerable information about how the central nervous system functions. Recent research by Karten at the University of New York at Stony Brook, using improved immunohistochemical techniques, shows that several peptides of the optic tectum (located in the roof of the midbrain) are distributed in layers. The peptides, including enkephalin, substance P, neuropeptides, bombesin, cholecystokinin, vasoactive intestinal polypeptide, and pancreatic polypeptide, were previously known to be present in the optic tectum, but their cellular distribution and the laminar nature of this structure were unknown. Discovery of these layered patterns of peptides was made initially in the brains of birds and amphibia; further study has demonstrated similar, though less elaborate, arrangements in the optic tectum of rabbits and cats. The functional reason for this arrangement must yet be determined.

Directional and orientational properties of neurons:

Considerable data indicate that early visual experience has a profound effect on development of the visual system. Visual deprivation in young animals or human infants is known to lead to visual deficits that become increasingly difficult to correct with increasing age of the human or animal. Animals have frequently been used to examine the effects of visual

deprivation. One experimental approach has been to rear cats under stroboscopic illumination, which prevents them from seeing moving objects. Cats that were reared under strobe lighting were also trained in a motion discrimination task in which a particular direction of motion was always "correct", a training that stimulated only certain types of neurons. Behavioral studies of these cats showed that they had a strong bias to the trained directions (velocity thresholds are much lower in this direction). Neurophysiological studies by Movshon of New York University revealed a bias that paralleled this behavioral preference: more cells responded to the trained direction than other directions. These results demonstrate an important correlation between the behavior of individual cortical cells and the overall visual behavior of the animal.

Discovery of a new visual area in the brain:

In order to understand how visual processing occurs, it is necessary to identify those portions of the brain that are important for vision. Recently, Graybiel of the Massachusetts Institute of Technology has described a "new" visually responsive area in the brain of cats that is quite far removed from most classically identified "visual areas." It is located at the side of the brain near the auditory and somatic sensory areas of the cortex in the anterior ectosylvian sulcus. This area responds only to visual stimulation and has been named the extrastriate visual area (EVA). Preliminary studies of EVA and the patterns of neuronal connections suggest that still other areas of the brain play functional roles in vision as well. Communication between various visually responsive areas of the cortex appear to be routed through the pulvinar region of the thalamus, rather than via direct transcortical connections.

Discovery and functional significance of anatomically identifiable patches or "blobs" in primate brain:

Recent reports from the laboratory of David Hubel at Harvard University, indicate that structural components, affectionately known as "blobs", have been seen in the brains of monkeys and humans. These blobs have been detected by stains that localize the activity of certain enzymes, including cytochrome oxidase, succinic semialdehyde dehydrogenase, and lactate dehydrogenase. The blobs are arranged in rows and are about 500 micrometers apart in patterns that correspond to the ocular dominance columns. Cells containing the blobs respond to diffuse light, especially diffuse red light, but their importance has not yet been determined. However, their localization along ocular dominance columns, the finding that damage to an eye renders the blobs shrunken and pale, and the fact that exposure of the animal's eyes to light causes the blobs to become labeled with radioisotopes designed to identify high levels of metabolic activity, all suggest that they may well be functionally important. They have been found in all primates studied, but not in nonprimates.

Animal models for amblyopia:

Suturing the eyelid shut in animals has been used for several years to induce amblyopia "lazy eye" and to produce a research animal model with characteristics similar to this common disorder seen in humans. However, lid suture differs from most cases of amblyopia that occur naturally in humans, because lid suture causes essentially complete deprivation of visual stimulation, while most amblyopia-causing conditions in humans allow for some visual stimulation, even if reduced or abnormal. Improved animal models are needed that more closely resemble the strabismic amblyopia that occurs in humans.

Esotropia can be produced in monkeys by surgery on one or more extraocular muscles, producing a misalignment of the eye. If this surgery is performed early in the development of the animal, amblyopia results. Research on these animals shows that cortical neurons driven by the deviating eye constitute less than 15 percent of the total, and binocular neurons are nearly absent. Research on strabismic amblyopia is likely to be assisted by the recent finding by Boothe at the University of Washington of a colony of monkeys with naturally occurring strabismus that can be studied without artificial alteration of the extraocular muscles.

Another procedure for generating amblyopia has been developed that consists of treating one eye of macaque monkeys with atropine. The visual image for near objects in that eye is then constantly blurred. Such treatment administered throughout the first six months of life produces a reproducible deficit in the vision of the treated eyes, though less drastic than seen after complete occlusion. Electrophysiological studies by Movshon of the central visual pathways of atropine-treated monkeys revealed significant differences from those seen for either lid-sutured animals or those with esotropia produced surgically. Neurons driven by the atropinized eye were not less numerous than those driven by the normal eye but, as seen for lid-sutured animals and those with surgically induced esotropia, binocularly-driven neurons were relatively rare. Neurons driven by the atropinized eye tended to have lower optimal and cutoff spatial frequencies than those driven by the normal eye and had lower contrast sensitivities.

Morphological studies of the visual processing system of atropine-treated animals revealed changes in specific types of cells (X-like cells) in a portion of the midbrain known as the dorsal lateral geniculate nucleus. Studies with the atropinized eyes may provide some insight as to what type and how much visual stimulation is needed for normal visual development, and what types of deprivation can lead to amblyopia. Atropine paralyzes the ciliary muscles, thereby preventing accommodation and making images appear fuzzy, particularly those of nearby objects. This would minimize the animal's visual stimulation, particularly near vision. The animals in this study were raised in cages in a normal animal colony room and thus would have been exposed to some visual stimulation at several meter where the atropine would be expected to have little or no effect on visual

acuity. The animals were known to be hyperopic at the initiation of atropine treatment, and the greater their hyperopia at the beginning of the treatment, the deeper the resulting amblyopia.

What causes stabilized images to disappear?:

Eye movements are a necessary part of vision. In addition to helping sary part of vision. In addition to helping individuals search and scan their environment to locate a target, they apparently are necessary to maintain visual sensitivity to a target. This has been demonstrated by special techniques that compensate for normal eye movements and stabilize an image on the retina; after a few seconds or minutes, most stabilized visual stimuli fade and disappear. How fast such stabilized images disappear has been shown by several investigators to depend on the contrast and luminance of the image; the higher the contrast and/or luminance, the longer the time required for the image to disappear. Recent studies by Kelly suggest that at least some types of stabilized images disappear because of the formation of a negative afterimage that cancels out the positive image. The effect does not appear to be caused by light bleaching of pigment in the photoreceptor cells, since the paler stabilized images fade faster than brighter ones or those of a higher contrast. If this is true, the effect would be caused by some post-receptor mechanism. However, this hypothesis has by no means been universally accepted. Studies are under way to prove or disprove the hypothesis and to learn more about what causes these images to disappear.

Pharmacological treatment of strabismus:

In some forms of strabismus, also known as cross-eye or walleye, one muscle of a pair is much stronger than its contralateral muscle and pulls the eye out of proper alignment with the other eye. Strabismus can sometimes be treated with prisms or corrective lenses, but at the present time surgery is generally required to correct the more extensive cases of the disorder. A new, nonsurgical experimental approach for treating strabismus is the the injection into extraocular muscles of botulinum, known as Oculinum for this treatment. Botulinum is the very toxic substance that can cause serious illness or death when a person consumes foods that have been improperly canned, but the toxic properties of this substance are being put to good use as a possible means of effectively and reasonably inexpensively treating strabismus.

The idea behind the therapy is to inject a tiny amount of botulinum into the stronger of a pair of muscles to weaken it and to give the weaker, contralateral muscle a chance to pull the eye into proper alignment. At present, 91 patients have been treated by Scott of the Smith-Kettlewell Institute of Visual Sciences. Once satisfactory dose levels were established, nearly all cases of horizontal strabismus have been significantly corrected.

The effect seems to be reasonably persistent, and cases have been followed now for up to three years. The drug appears to be useful for preventing contracture of tight muscles, treating spastic contracture, and for treating ocular disorders associated with cerebral palsy. Further research is being performed to determine its efficacy for other ocular muscle disorders and vertical forms of strabismus. The drug and treatment apparatus are being provided to other investigators to permit independent assessment.

Other uses are being found in strabismus therapy for the injection of a paralyzing substance into an extraocular muscle. Since there are six extraocular muscles for each eye, it is sometimes important to determine which muscle is the offending one. Such determinations could be made before surgery by injecting a muscle with a substance that would temporarily weaken it, and then determining what the effect of this muscle weakening is on ocular alignment. This approach is being tested by the injection of the anesthetic xylocaine into an extraocular muscle, using techniques that are very similar to those used for injection of the longer-acting botulinum.

Future Needs:

Basic neurobiological work in this program is quite strong, as exemplified by the awarding of the 1981 Nobel Prize for Physiology or Medicine to two long-time NEI grantees, Torsten Wiesel and David Hubel, for their studies of the developing structure and function of the visual cortex. Future progress in our understanding of the visual system may come most rapidly from studies of the molecular basis of cellular functioning and growth. Extension of sophisticated behavioral techniques to the clinical diagnosis of visual disorders in adults and, especially, young children will also be emphasized. Better characterization of the nature of amblyopic vision, particularly with respect to prognosis for successful treatment will be encouraged.

Disturbances of ocular motility are an important source of sensory deficits, but little is known about either the normal development of eye movements or the mechanisms of eye movement disorders. There is a real need to develop techniques for easily measuring the eye movements of infants and young children as well as those of adults with a variety of neuro-ophthalmic disorders. Just as for sensory processes, studies of neurotransmitter systems involved in eye movements will be encouraged with a view toward pharmacological intervention in disorders.

Since most attention in the past has been given to the control of conjugate eye movements, in which the eyes move in the same direction, increased emphasis is now necessary for research on vergence eye movements (movement of the eye toward or away from one another when looking at nearby or far away objects) and mechanisms of accommodation, in which the shape of the lens is changed by eye muscles to focus on objects at different distances.

Research on the varying etiologies of strabismus and alternative methods of treatment will be a high priority for this program. Emphasis will be placed on developing techniques to predict better the outcome of surgery, thereby increasing its accuracy and reducing the reoperation rate, which now stands at 25 percent. Drug treatments for strabismus will also be pursued as an alternative to surgery.

An accelerated pace of research on the etiology and mechanisms of myopia will be fostered, using both animal models and physiochemical approaches. A basic understanding of the development of this widespread disorder is necessary before true preventive techniques or therapy other than optical correction is possible.

There is a continuing, unmet need for research on the visual characteristics of individuals with specific types of visual impairments. Development and, especially, evaluation of optical and electronic aids for visually impaired persons must also be stimulated. To the extent that is possible, given the heterogeneity of the visually impaired population, generic technologies, rather than idiosyncratic, must be developed.



OFFICE OF BIOMETRY AND EPIDEMIOLOGY



ANNUAL REPORT
NATIONAL EYE INSTITUTE
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REPORT OF THE CHIEF, OFFICE OF BIOMETRY AND EPIDEMIOLOGY
Fred Ederer

The Office of Biometry and Epidemiology has three main functions: research, education, and consultation.

Research is the dominant function. It is the Office's mission to plan, develop, and carry out studies on human populations concerned with the causation, prevention, and treatment of eye disease and vision disorders, with emphasis on the major causes of blindness. This includes studies of incidence and prevalence in defined populations, prospective and retrospective studies of risk factors, natural history studies, clinical trials, genetic studies, and studies to evaluate diagnostic procedures.

Education: The Office carries out a program of education in biometric and epidemiologic principles and methods for the vision research community. This consists of courses, workshops, a pre- and post-residency fellowship program for ophthalmologists, publications, and consultation and collaboration on research.

Consultation: The Office provides biometric and epidemiologic assistance to National Eye Institute intramural and extramural staff and to vision research workers elsewhere. The assistance ranges from referral to appropriate consultants to collaboration as co-investigator.

Research

Clinical Trials. Three contract-supported clinical trials on the treatment of diabetic retinopathy are in progress. These are the Diabetic Retinopathy Study (DRS), the Early Treatment Diabetic Retinopathy Study (ETDRS), and the Diabetic Retinopathy Vitrectomy Study (DRVS).

The DRS Research Group has published eight major papers and made numerous presentations. The study's data base has been organized in a fashion that is usable by any interested investigator and is now available for this use. Continuing data analysis should result in preparation and publication of several additional major papers in the coming year.

The ETDRS was designed to provide a better understanding of the optimum time to use photocoagulation in the course of diabetic retinopathy. Patients with macular edema, preproliferative, or proliferative retinopathy are being studied. Three forms of photocoagulation treatment, ranging from a restricted focal treatment to a full scatter, are being assessed. In addition, the study is evaluating the effect of daily administration of aspirin on the incidence of microvascular and macrovascular complications. The study also provides the opportunity to investigate factors that are associated with the progression of disease. As of July 1982, nearly 1,850 treatment allocations had been issued and over 1,590 patients had been treated, with recruiting likely to last until the end of 1983.

The DRVS has succeeded in recruiting over 600 patients whose vision has been reduced by hemorrhage into the vitreous (group H) and over 300 patients who still have useful vision but with serious risk of complications that often lead to retinal detachment (group NR). Half of the eligible eyes in both groups are being randomized to prompt vitrectomy, and half to vitrectomy one year later, if still indicated (in group H), or to "traditional" care (in group NR). Recruitment will likely terminate not later than April of 1983. The results of the randomized part of the study will not be available for some time, but the first two years of the natural history component of the study is now being summarized for publication.

A fourth, grant-supported clinical trial, the Prospective Evaluation of Radial Keratotomy Study (PERK), designed to evaluate a surgical procedure to correct myopia, is now in its patient recruitment phase. Recruitment is expected to be completed in early 1983. Patients will be followed for four years. Dr. Robert Sperduto is the Institute's Scientific Project Director for the study and serves on all major PERK committees.

Epidemiology. The Office organized and conducted a Symposium on the Epidemiology of Eye Diseases and Visual Disorders in Bethesda, Maryland, on June 10-11, 1982. Fred Ederer, Daniel Seigel, and Dean Krueger (George Washington University) constituted the Scientific Program Committee. Twenty-nine papers, including invited literature reviews of five major causes of visual impairment, were presented along with invited and spontaneous discussions. The Symposium, attended by more than 100 scientists from the United States and abroad, was the first national or international meeting on this subject ever held. Selected papers from the Symposium will be published in a single issue of the American Journal of Epidemiology in the Spring of 1983. Mr. Ederer, Mr. Podgor, Dr. Ferris, Dr. Sperduto and Mrs. Hiller all presented papers. Mr. Ederer also served as discussant of a session on open-angle glaucoma and as a Symposium Summary discussant.

Collection of data in the pilot study for a national Visual Acuity Impairment Survey (VAIS) is nearing completion. The primary objective of the national VAIS will be to determine the prevalence of visual acuity impairment, by cause, in representative samples of the population of large metropolitan areas of the United States. Of nearly equal importance is the opportunity to conduct case-control studies of risk factors for impairment due to each of the major diseases, within the VAIS population. Diseases which are expected to be identified as major causes of impairment include senile cataract, macular degeneration, amblyopia, diabetic retinopathy and open-angle glaucoma.

Major issues to be resolved in the pilot study are whether (1) home screening of visual acuity with pinhole correction by lay interviewers can correctly identify nearly all persons whose acuity is 20/50 or worse; (2) sufficiently large proportions of persons with impaired vision and of age-matched controls will attend an eye clinic for comprehensive ophthalmic examination; and (3) the clinic procedures and forms for recording data are satisfactory. Interim analyses of data collected to date indicate that the home screening process is successful and the clinic procedures and data forms are satisfactory. Substantial difficulties are being encountered in obtaining adequate numbers of clinic examinations of subjects 75 years of age and older.

A final report on the pilot study, with recommendations regarding the feasibility of a national VAIS, will be prepared and completed in early 1983.

Drs. Seigel, Sperduto and Ferris collaborated in two short scientific communications on the reported role of aspirin in retarding cataract formation. They critically reviewed publications by Cotlier on this subject and reported data from the Framingham Eye Study in which no association between aspirin use and lens opacities was present. Their two publications provide some counterweight to the widespread attention which this aspirin hypothesis has received in the lay press.

Dr. Seigel and Mr. Podgor co-authored a paper on life table analysis with paired data. This is particularly important for eye research, where the problem frequently arises.

Mrs. Hiller and Dr. Sperduto, and Professor Krueger from the George Washington University, published a study on pseudoexfoliation, intraocular pressure, and senile lens changes in the Framingham Eye Study. They found that prevalence rates of pseudoexfoliation increased with age, from 0.6% for age 52-64 to 5.0% for ages 75-85, and were similar to those reported in Norway where the condition is thought to be especially common. Pseudoexfoliation was found to be associated with higher intraocular pressure levels and more frequent senile lens changes, but the latter relationship was not statistically significant.

Using data from the 1971-1972 Health and Nutrition Examination Survey (HANES), Mrs. Hiller, Dr. Sperduto, and Professor Krueger published a paper demonstrating that intraocular pressure is positively associated with systolic blood pressure, age, and amount of iris pigmentation. The association between amount of iris pigmentation and intraocular pressure had not been previously reported.

The HANES asked subjects whether they had "trouble with...vision even when wearing glasses or contact lenses" and also measured their central distance visual acuity with usual corrective lenses. Mrs. Hiller and Professor Krueger found that the question had low sensitivity for impairment of visual acuity. A paper is being published in the American Journal of Public Health.

In a multivariate analysis of HANES data Mrs. Hiller, Dr. Sperduto, and Mr. Ederer found that senile cataract was positively associated with increasing age, increasing UV-B radiation and decreasing number of years spent in school, and was more common among blacks, diabetics and rural dwellers. This material was presented at the National Eye Institute Symposium on Eye Disease Epidemiology in June 1982 and is being submitted for publication to the American Journal of Epidemiology.

Dr. Sperduto, Dr. Seigel, Ms. Jean Roberts and Mr. Michael Rowland used data from the 1971-1972 National Health and Nutrition Examination Survey to estimate myopia prevalence rates for people in the United States between age 12 and 54. Lower prevalence rates were found for men than women and for blacks than whites. Myopia prevalence rose with family income and educational level.

Dr. Sperduto collaborated with Dr. M. Cristina Leske of the State University of New York at Stony Brook, in preparing a paper that reviewed the epidemiology of senile cataract. The paper is being submitted for publication in the American Journal of Epidemiology.

Mr. Podgor and Mr. Ederer have continued to work with Dr. Leske in a study of glaucoma screening methods using the Framingham Eye Study data. This material was presented at the May 1982 ARVO meeting and a manuscript is being prepared for publication. They have also estimated the incidence of common eye conditions from Framingham Eye Study prevalence data using a method they had developed for glaucoma. Mr. Podgor presented this work at the NEI Epidemiology Symposium, and the material is being submitted for publication in the American Journal of Epidemiology. Mr. Podgor and Dr. Leske have also investigated associations of intraocular pressure, blood pressure, and visual field defects in the Framingham Eye Study population. They presented papers on this topic at the ARVO meeting and at the NEI Epidemiology Symposium. Mr. Podgor and Dr. Robert N. Frank, Kresge Eye Institute, have completed work on an extension of a study of retinopathy in juvenile-onset diabetes of short duration. This material was also presented at the ARVO meeting and the NEI Epidemiology Symposium. The paper describing this work has been accepted for publication.

Mr. Podgor collaborated with Drs. de Monasterio and Jaffe of the NEI Clinical Branch on a study of retinal aging and blue-sensitive cone function in humans. Results of this work were presented at the ARVO meeting.

Drs. Seigel, Sperduto, and Milton participated in a WHO/NEI informal workshop on cataract epidemiology.

Dr. Milton is acting project officer for the Clinical Research Center for Prevention of Malnutritional Blindness, a collaborative research effort between the NEI and the National Institute of Nutrition (NIN), Hyderabad, India. Projects of the Center will include a case-control study of risk factors in severe xerophthalmia in preschool children, as well as special biochemical and clinical studies of vitamin A deficiency and xerophthalmia. Following Dr. Milton's visit to Hyderabad in January, the establishment of the Center was given final approval by the NIH and the Indian Council for Medical Research. The NEI is purchasing equipment for the Center under a Participating Agency Service Agreement (PASA) with AID. Delivery of equipment and activation of projects are expected in late 1982.

Education and Consultation

Dr. Kupfer, Dr. Ferris, and Mr. Ederer participated as faculty in the third of a series of annual courses on epidemiologic and biostatistical approaches to clinical vision research. Along with university colleagues, they presented a three-day course at Sarasota, Florida, to clinical investigators prior to the 1982 ARVO meeting. Attendance was excellent, and written evaluation indicated that the course material was quite appropriate. A fourth course is planned for 1983.

Dr. Ferris, Dr. Seigel, Mr. Ederer, and Dr. Matthew Davis (University of Wisconsin) conducted a three-hour course on methods of vision research at the annual meeting of the American Academy of Ophthalmology.

Mr. Ederer is a member of the Editorial Board of the American Journal of Ophthalmology and Dr. Seigel is a member of the Editorial Board of Archives of Ophthalmology. Both Mr. Ederer and Dr. Seigel are Associate Editors for the American Journal of Epidemiology. Mr. Ederer is a member of the Board of Directors of the Society for Clinical Trials and of the American College of Epidemiology.

In March 1981, NIH held an international symposium "Current Concepts in Biometry and Epidemiology" in honor of Jerome Cornfield, a renowned biostatistician and former consultant to NEI who died in 1979. The proceedings, including a paper by Mr. Ederer, and an introduction by Dr. Seigel, have been published as a supplement to the March 1982 issue of Biometrics. Dr. Seigel chaired and Mr. Ederer served on both the planning committee for the symposium and the editorial board for the publication.

The National Eye Institute is represented through Dr. Milton on the NIH Advisory Committee for Computer Usage, Dr. Seigel on the NIH Clinical Trials Committee, and Mr. Ederer on the NIH Epidemiology Committee.

Dr. Seigel provided consultation to Drs. Kupfer and Meyers on a clinical trial to retard progression of senile macular degeneration. He worked with Dr. Kupfer to develop a protocol for a clinical trial of drug therapy in diabetic patients with no or very early retinopathy.

Dr. Seigel and Dr. Sperduto met with Dr. Geller, NEI Associate Director for Extramural Programs, to provide consultation to Dr. Donald Sanders, University of Illinois, Eye and Ear Infirmary, on the analysis of clinical data on radial keratotomy surgery. Dr. Seigel worked with Dr. Israel Goldberg, NEI Chief of the Retinal and Choroidal Diseases Branch, to develop an information packet for use upon release of the findings of the Senile Macular Degeneration Study, an NEI grant supported, multicenter clinical trial.

Dr. Milton participated in a workshop for Helen Keller International on the evaluation of the HKI blindness program in Haiti. He assisted Dr. Lawrence Brilliant, University of Michigan and the SEVA Foundation, in reviews of results from the Nepal Blindness Survey. Together with Mr. St. Palley of the Biometry Section, he assisted Dr. Muriel Kaiser, Clinical Branch, in the establishment of a computer system for data management of pigment dispersion syndrome data.

Mr. Podgor consulted with members of the Clinical Branch on the design of a study of visual field screening methods.

Dr. Sperduto served as a temporary advisor to the World Health Organization's Program Advisory Group. Dr. Sperduto and Mrs. Hiller also serve as consultants to the Cataract Panel of the National Advisory Eye Council Program Planning Subcommittee.

Dr. Ferris has been appointed to the Data and Safety Monitoring Committee for the Branch Vein Occlusion Study, another grant-supported multicenter clinical trial.

Dr. Seigel received the Superior Performance Award, the highest Public Health Service honor, in recognition of his work in national health studies and in statistical methods.

Dr. Milton participated in review of grant applications and contract proposals for the National Cancer Institute and for the Visual Sciences A Study Section, Division of Research Grants.

Dr. Milton presented a lecture on the epidemiology of cataract in Punjab at the Wilmer Institute, Johns Hopkins University.

Publications

Office of Biometry and Epidemiology

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2. Ederer F: Jerome Cornfield's contributions to the conduct of clinical trials. In Proceedings of Current Topics in Biostatistics and Epidemiology, A Memorial Symposium in Honor of Jerome Cornfield. Biometrics 38(Supplement):25-32, 1982.
3. Ferris FL, Kassoff A, Bresnick GH, Bailey I: New visual acuity charts for clinical research. Am J Ophthalmol 94:91-96, 1982.
4. Ferris FL, Sperduto, RD: Standardized illumination for visual acuity testing in clinical research. Am J Ophthalmol 94:97-98, 1982.
5. Hiller R, Sperduto RD, Krueger DE: Pseudoexfoliation, intraocular pressure, and senile lens changes in a population-based study. Arch Ophthalmol 100:1080-1082, 1982.
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8. Chatterjee A, Milton RC, Thyle S: Prevalence and aetiology of cataract in Punjab. Br J Ophthalmol 66:35-42, 1982.
9. Seigel D, Podgor MJ: A sign test for significance of differences in survivorship curves from paired truncated data. Controlled Clin Trials 3:69-71, 1982.
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12. Sperduto RD, Seigel D, Roberts J, Rowland M: The prevalence of myopia in the United States. Arch Ophthalmol (in press).
13. Hiller R, Krueger DE: Validity of a survey question as a measure of visual acuity impairment. Am J Public Health (in press).
14. Frank RN, Hoffman WH, Podgor MJ, Joondeph HC, Lewis RA, Margherio RR, Nachazel DP, Weiss H, Christopherson KW, Cromin MA: Retinopathy in juvenile-onset type I diabetes of short duration. Diabetes (in press).



CONTRACT NARRATIVE

Fifteen Clinical Centers plus a Coordinating Center at the University of Maryland School of Medicine, Baltimore, Maryland, and a Fundus Photograph Reading Center at the University of Wisconsin, Department of Ophthalmology, Madison, Wisconsin

Title: Diabetic Retinopathy Study (DRS)

Principal Investigator: Matthew D. Davis, M.D. (Study Chairman)

Current Fund Allocation: \$102,942 (Coordinating Center only) for fiscal year 1982.

Objectives: The Diabetic Retinopathy Study (DRS) was a multicenter clinical trial to evaluate the efficacy of photocoagulation, (argon laser and xenon arc) in the treatment of proliferative diabetic retinopathy. This randomized, controlled study involves 1,758 patients enrolled at 15 medical centers.

Major Findings: Photocoagulation with either argon laser or xenon arc, as used in the study, is effective in reducing the risk of severe visual loss and in inhibiting the progression of retinopathy. These effects were apparent in all stages of diabetic retinopathy studied: proliferative, severe nonproliferative, and background. Also found were some deleterious effects of treatment, namely small losses of visual acuity and constriction of the peripheral visual field.

Significance to Biomedical Research and the Program of the Institute: Diabetic retinopathy, uncommon only a few decades ago, is now a leading cause of blindness and visual disability in the United States. There is a critical need to find and evaluate scientifically treatments which will reduce the risk of blindness or visual impairment from the ocular complications of diabetes. This study has shown that photocoagulation is effective in reducing severe visual loss in eyes with proliferative diabetic retinopathy.

Proposed Course: Follow-up of all surviving DRS patients terminated on May 31, 1979. The study data have been edited and data tapes have been furnished to the National Eye Institute. Eight major papers on the results have been published and further analyses are under way. A final report on the results of long-term follow-up is being prepared. In addition, papers are being prepared on the following topics: comprehensive assessment of risk factors for severe visual loss, mortality in proliferative diabetic retinopathy, effects of photocoagulation treatment on diabetic macular edema, and association of renal disease and diabetic retinopathy.

NEI Research Program: Retinal and Choroidal Diseases--Diabetic Retinopathy, Sickle Cell Retinopathy, and Other Vascular Abnormalities

Publications:

The Diabetic Retinopathy Study Research Group: Photocoagulation treatment of proliferative diabetic retinopathy: A short report of long range results. Diabetic Retinopathy Study (DRS) Report Number Four. Excerpta Medica 789-794, 1980.

The Diabetic Retinopathy Study Research Group: Photocoagulation treatment of proliferative diabetic retinopathy: Relationship of adverse treatment effects to retinopathy severity. Diabetic Retinopathy Study (DRS) Report Number Five. Dev Ophthal 2:248-261, 1981.

CONTRACT NARRATIVE

Thirteen Clinical Centers, plus a Coordinating Center at the University of Minnesota, Minneapolis, Minnesota and a Fundus Photograph Reading Center at the University of Wisconsin, Department of Ophthalmology, Madison, Wisconsin

Title: Diabetic Retinopathy Vitrectomy Study (DRVS)

Principal Investigator: Matthew D. Davis, M.D. (Study Chairman)

Current Fund Allocation: \$754,708 for fiscal year 1982.

Objectives: The DRVS is a multicenter clinical trial to:

- a. Evaluate vitrectomy performed in the first six months after severe vitreous hemorrhage secondary to diabetic retinopathy compared to the more usual practice of waiting twelve months after vitreous hemorrhage to remove the vitreous.
- b. Evaluate vitrectomy in eyes with good vision but with severe proliferative retinopathy and poor prognosis before vision is lost through hemorrhage or retinal detachment.
- c. Study the natural history of severe proliferative diabetic retinopathy.

Major Findings: As of July 1982, a total of 562 eyes with severe hemorrhage had been randomized to early or deferred vitrectomy. Follow-up continued on the 777 eyes recruited in the natural history study, where recruiting has stopped. A total of 307 eyes have been randomized in group NR.

Significance to Biomedical Research and the Program of the Institute: Diabetic retinopathy is one of four major causes of adult blindness and differs from the other three (macular degeneration, glaucoma, cataract) in that it affects a younger population. Vitrectomy has the theoretical potential of removing the "scaffolding" on which abnormal new vessels can develop, fibrous tissue can form, and retinal detachment can occur. It is important to determine when such intervention is most likely to deter this process, and reduce the incidence of loss of vision. This presents an ideal opportunity for the National Eye Institute to mobilize scientific talents to answer a significant medical question.

Proposed Course: Consideration is being given to setting a date for termination of recruiting. The date likely to be selected is April of 1983. The number of patients accrued as of that date should nearly meet the goals originally established for group NR, but will fall short of the goal for eyes with severe hemorrhage. The slight loss of precision that may result is preferred to undue delay in publication of study findings.

A manuscript on the first two years of follow-up in the natural history study is being prepared.

NEI Research Program: Retinal and Choroidal Diseases--Diabetic Retinopathy, Sickle Cell Retinopathy, and Other Vascular Abnormalities

Publication: None



CONTRACT NARRATIVE

Twenty-three Clinical Centers, plus a Coordinating Center at the University of Maryland School of Medicine, Baltimore, Maryland; a Fundus Photograph Reading Center at the University of Wisconsin, Department of Ophthalmology, Madison, Wisconsin; a Central Laboratory at the Centers for Disease Control, Atlanta, Georgia; and an Electrocardiogram Reading Center at the University of Minnesota, Minneapolis, Minnesota

Title: Early Treatment Diabetic Retinopathy (ETDRS)

Principal Investigator: Dr. Lloyd Aiello (Chairman)

Current Fund Allocation: \$3,592,681 for fiscal year 1982.

Objectives: The Early Treatment Diabetic Retinopathy Study (ETDRS) is a multicenter, randomized clinical trial, the main goals of which are:

- a. To determine whether treatment of early stages of proliferative and nonproliferative diabetic retinopathy with or without macular edema by aspirin and/or prompt photocoagulation is effective in decreasing the rate of development of known retinopathy risk factors and/or the development of severe visual loss when compared to placebo or deferred photocoagulation.
- b. To determine the optimum time to initiate photocoagulation treatment in diabetic retinopathy.
- c. To monitor closely the effects of diabetes mellitus and/or of photocoagulation on visual function.
- d. To develop natural history data that can be used to develop (identify risk factors) and test etiologic hypotheses in diabetic retinopathy.

Major Findings: As of July 2, 1982, 2,393 patients had started qualifying visits for this study with 2,030 completing this visit. A total of 1,850 treatment allocations have been issued and 1,590 patients have been treated. The rate of recruitment is continuing to increase and it is expected that each clinic will recruit, on the average, five patients per month up to October of 1983. Four new clinics were added in May 1982 to aid in meeting the recruitment goal.

Significance to Biomedical Research and the Program of the Institute: The National Eye Institute regards fostering careful evaluation of new and widely used ophthalmic treatments as an essential element in its mission. This study represents an extension of the Institute's interest in preventing visual impairment of patients with diabetes.

Proposed Course: Follow-up of all ETDRS patients is planned for five years. Monitoring of accumulated data is performed at quarterly intervals.

NEI Research Program: Retinal and Choroidal Diseases--Diabetic Retinopathy, Sickle Cell Retinopathy, and Other Vascular Abnormalities.

Publications: None



CONTRACT NARRATIVE

Three Clinical Centers, plus a Coordinating Center at the University of Minnesota, Minneapolis, Minnesota; the Census Bureau; and a Fundus Photograph Reading Center at the Office of Biometry and Epidemiology, National Eye Institute, Bethesda, Maryland

Title: Visual Acuity Impairment Survey (VAIS) Pilot Study

Principal Investigator: Fred Ederer (Project Officer)
Richard Mowery (Deputy Project Officer)

Current Fund Allocation: \$6,489 for fiscal year 1982.

Objectives: The Visual Acuity Impairment Survey Pilot Study is a planned multicenter epidemiological study of the prevalence of central distance visual acuity impairment in the United States and of the eye diseases responsible for impairment. The main goals of the 1-year study are:

- a. To determine the feasibility of the VAIS.
- b. To pretest home interview screening procedures and clinic examination procedures.
- c. To gather information that will help to plan the full study.

Major Findings: The VAIS Directors Committee met in December 1981 and in June 1982 to review the progress of the pilot study and to evaluate the procedures used in the home screening and in the clinic examination. At both meetings, modifications were made to the study protocol to enhance subject recruitment. As of July 16, 1982, the Census Bureau completed home screening for 1,615 subjects who were 25 years of age or older. Approximately 217 of the subjects were eligible for clinic examination, based on the study selection criteria, and were referred to the clinic. The three pilot study clinics had completed clinic or home examinations on 115 of the 217 eligible subjects.

Significance to Biomedical Research and the Program of the Institute: The Visual Acuity Impairment Survey originated from the Institute's past involvement with the Model Reporting Area of Blindness, the Health and Nutrition Examination Survey, and the Framingham Eye Study. These studies attempted to measure the frequency of eye diseases or of visual impairment but were limited in scope or assurance of quality, or were hampered by logistical problems. The VAIS represents an extension of the Institute's interest in epidemiologic research and in gathering high quality population-based data to be used in program planning and for public information. The Study further offers the opportunity to introduce vision researchers to epidemiologic concepts and methods.

Proposed Course: Home screening by Census Bureau interviewers will be completed by August 21, 1982. The three clinics will continue recruiting and examining eligible subjects through December 1982. All data collected at home and in the clinic are sent to the Coordinating Center in Minnesota for processing and analysis. The Coordinating Center will complete data processing and analysis by March 1983 and will assist the Institute staff in preparing a recommendation concerning the feasibility for a main study.

The recommendation will be presented to the NEI Director in April 1983.

NEI Research Program: Multiprogram

Publications: None

OFFICE OF PROGRAM PLANNING, ANALYSIS, AND EVALUATION



ANNUAL REPORT
NATIONAL EYE INSTITUTE
October 1, 1981 - September 30, 1982

REPORT OF THE CHIEF, OFFICE OF PROGRAM PLANNING, ANALYSIS, AND EVALUATION
Julian M. Morris

Planning and Evaluation Section

In FY 1982 the Office completed organizing and conducting a series of Panel and National Advisory Eye Council Program Planning Subcommittee meetings which will culminate in the publication of Vision Research--A National Plan: 1983-1987. During the past year the Office worked with other NEI staff offices and the Subcommittee to prepare the "Executive Summary" and Volume One, "Report of the National Advisory Eye Council" of the Plan and with staff and chairmen and members of the planning panels to prepare final drafts of the six panel reports that constitute Volume Two of the Plan. In particular, OPPAE staff collaborated with NEI Extramural Program Directors to edit, and in some cases redraft, these reports for clarity, scientific accuracy, and conformance to NEI policy. In preparation for publication of Volume Three, "Support for Vision Research," the Office completed organizing and reviewing all NEI program information and attainable data on vision research projects supported by organizations other than the NEI.

The Office worked with a publication designer and Government Printing Office staff to develop a system for computerized typesetting of virtually all 2,200 pages of the Plan's manuscript. The Office prepared an exhibit and Mr. Morris made a presentation on the new planning report at the 1982 Annual Meeting of the Association for Research in Vision and Ophthalmology in Sarasota, Florida.

As in past years, the Office coordinated the development of NEI program evaluations, prepared the annual NEI Evaluation Plan, and wrote Impact Statements for completed evaluation projects. The Office also drafted the NEI contribution to the NIH Research Plan (FY 1983-1986), and completed a program performance summary of the NEI Cataract Program, a document that was cited by OPPE/NIH as a model for other Institutes' programs. The Office assisted the Extramural and Collaborative Programs Branch in developing a proposal for an evaluation of the NEI Small Grants Program.

During the past year, Mr. Morris advised the Director, NEI, concerning possible international program planning strategies for the World Health Organization's (WHO) Prevention of Blindness Programme and attended a meeting of a WHO working group in Geneva to discuss program planning with the Programme Manager. He subsequently advised the Director, NEI, of his findings and made recommendations to him concerning further action.

Mr. Morris continued to serve as NEI Prevention Coordinator and the Office responded to numerous requests from OD and DHHS for data and descriptive materials concerning NEI prevention activities. Mr. Morris

conducted a briefing on the NEI planning process for Glenna M. Crooks, Ph.D., newly appointed Deputy Assistant Secretary for Health Planning and Evaluation.

As NEI Legislative Liaison, Mr. Morris kept NEI staff informed of relevant legislative developments. The Office coordinated NEI comments on various provisions of the Health Research Act of 1982 and prepared background materials for the annual briefing of the NIH Director concerning NEI programs, plans, and issues in preparation for the FY 1983 Appropriations Hearings.

During FY 1982 the Office directed a contract for Professor Teh-wei Hu of Pennsylvania State University to prepare a report, "The Economic Costs of Visual Disorders and Blindness in the U.S., 1981."

Mr. Morris served as Head of the NEI Office Automation Task Force which evaluated various word processing equipment and made a preliminary recommendation to select one system for a pilot trial within the NEI Budget Office. He also served as a member of the NEI Institute Review Board for Clinical Branch research protocols and assisted several intramural investigators in drafting informed consent documents prior to review by the Board.

In addition, the Office has contributed to, commented upon, or coordinated NEI's contributions to the following reports, meetings, or requests for information, data, or review:

- o Aging-related projects sponsored by the NEI for the five-year period, 1976-1980.
- o Surgeon General's Request for Information on Research Related to Long-Term Care.
- o "Developments in Aging" report.
- o Inventory of Federally Supported Research on Aging.
- o NEI support of genetic research active in FY 1982.
- o Drug Abuse Research Project Inventory System for the National Institute on Drug Abuse.
- o Annual Drug Abuse Report from the Secretary to the President and Congress.
- o World Health Organization's "International Nomenclature of Disease."
- o Indian Health Service Annual Survey for the Health Services Administration.
- o NIH Director's Advisory Council Subcommittee and NIH Working Groups on Planning Strategies.
- o NEI procedure for B/A/D reporting.

- o Fifth Annual Science and Technology Report.
- o Draft Guide for Classifying NIH Planning, Evaluation, and Legislative Analysis Positions.
- o Report of the Pharmaceutical Manufacturers Association Commission on Drugs for Rare Diseases.
- o Interagency Technical Committee Report of Federally Supported Research Related to Heart, Lung, and Blood Diseases and Blood Resources Update for FY 1981.
- o NEI Extramural Support of Blood and Blood-Related Research in FY 1981.
- o Draft GAO Report on Progress in Federal Human Nutrition Research.
- o Cancer-Related Research in FY 1981 and Estimated Support for FY 1982.
- o Office of Science and Technology Policy Request for Report on NIH Biomaterials Research.
- o Toxicology-related research to be included in the National Toxicology Program.

Again this year, the Office coordinated the preparation and supervised the publication of the NEI Annual Report.

Program Analysis Section

During FY 1982 the Program Analysis Section effected major changes in personnel, computer software and equipment, and office configuration to provide faster and better service to a variety of NEI users. Specifically:

1. PAS hired and trained a computer programmer, a computer assistant, and a fiscal assistant.
2. PAS streamlined its procedures for maintaining the grants, contracts, intramural, and training files. These data sets are now updated through prompts from user-friendly procedures rather than through multiple program changes and submissions. These new procedures yield greater reliability and require far less operating time.
3. Using these data management procedures, PAS has brought the NEI contracts, intramural, and training files up to date; the grant file has always been kept current.
4. PAS has installed a totally new "I2I" query system for generating reports from its data bases. The "I2I" system has greatly improved the turnaround time for most requests, permitting simple requests

to be processed within minutes if necessary. In fact, this new system allows most requests to be processed in about a quarter of the time that would be required using the old IRS system. This system also provides automatic management and documentation of all requests that are performed by PAS, and, pending training of NEI staff, will allow interested NEI users to program their own requests using simple commands.

5. PAS has developed and implemented a tasking system which automatically performs production tasks on the computer with the frequency required (i.e., weekly, quarterly, annually).
6. PAS has reviewed all production reports regularly produced for NEI staff. Using the new query system, the tasking system, and a custom data base, we are now in the process of streamlining, enhancing, and cataloging these reports to serve the NEI staff more efficiently, and to allow for timely modifications when user needs change.
7. PAS has reconfigured its office space and purchased new computer terminals and microfiche equipment to provide an enhanced yet more economical data management capability.
8. PAS has performed systems work and coded the data base of the entire text of Vision Research--A National Plan: 1983-1987 for computerized typesetting. PAS has also extracted, coded, and modified the NEI grants, contracts, and intramural project data bases for use in Volume Three of the National Plan and has created a data base of vision research projects supported by organizations other than the NEI.
9. PAS has refined the coding system used for program priority tracking in the NEI grants data base. It has also improved the computer program that is used to query the grants file based on scientific coding (SCORE), allowing virtually instantaneous retrieval of information on which research is being supported in different subject areas.

OFFICE OF SCIENTIFIC REPORTING

ANNUAL REPORT
NATIONAL EYE INSTITUTE
October 1, 1981 - September 30, 1982

REPORT OF THE CHIEF, OFFICE OF SCIENTIFIC REPORTING
Marsha S. Corbett

Historically, prevention of blindness and eye disease has been the long-range goal of the National Eye Institute (NEI) scientific reporting program. In FY 1982, prevention activities assumed even greater importance as prevention of needless loss of vision became the immediate short-term objective of several OSR projects. Outlined below are efforts made to disseminate sight-saving information harvested from two NEI-supported clinical trials. Examples of other projects in which blindness prevention is the goal are also discussed.

Diabetic Retinopathy

During the past fiscal year, the Office of Scientific Reporting prepared a diabetic retinopathy information campaign proposal which was accepted by the Secretary of HHS as one of his prevention initiatives. The overall campaign objective is to prevent needless loss of vision from diabetic retinopathy. Information on how to do this is being disseminated to diabetics, their families and friends; and to health care professionals, including ophthalmologists, optometrists, primary care physicians, nurses, and paramedics.

Components of the message for health care professionals include:

- o Sight-threatening complications of diabetes are more common than is generally realized.
- o The longer the patient has diabetes, the more likely he or she is to lose some vision.
- o Early signs of diabetic eye disease can be detected.
- o Appropriate use of photocoagulation can reduce the risk of going blind by 60 percent.
- o Diabetic patients should be examined to determine whether they need photocoagulation to prevent visual loss, or they should be referred to some other professional who can make this determination.
- o Health care professionals should provide information on diabetes-associated problems to their patients who have this disease.

The message to be communicated to laymen is as follows:

- o Diabetes can cause eye problems, including blindness.
- o But there is an effective treatment that can prevent loss of vision.

- o Diabetics should ask their doctor whether they need this treatment.

A new NEI publication Diabetes and Your Eyes has proved to be an important vehicle for conveying these messages. Fifty thousand brochures were ordered and few are left, despite the fact that implementation of the campaign proposal began only a few months ago. Word-of-mouth publicity about the booklet created a heavy demand for the booklet in the private sector among organizations willing to distribute the brochure at no cost to the National Eye Institute. Further efforts in this regard are being made to insure widespread dissemination to diabetics and their families at the lowest possible cost. In addition, the National Diabetes Information Clearinghouse agreed to handle mailing of the brochures in response to requests stimulated by media coverage of the topic. For example, following publication of an article on preventing blindness from diabetic retinopathy in the National Enquirer, one to two hundred requests a day for the brochure were received for several weeks. Reprints of the brochure have been ordered to meet the growing demand for this booklet.

Laymen and health care professionals alike are also learning how to prevent visual loss from diabetic retinopathy in a variety of other newspaper, magazine, and journal articles stimulated and/or drafted by the NEI; in exhibits at meetings of health care providers, health fairs, and continuing medical education courses; through speeches by Secretary Schweiker and other HHS officials; via televised features, public service announcements, brochures, Presidential proclamations; and through Centers for Disease Control, hospital-based, and Bureau of Indian Health diabetes programs. In addition, several private voluntary organizations are cooperating with OSR on the development of information materials and public health education campaigns aimed at preventing blindness from diabetic retinopathy.

Senile Macular Degeneration

During FY 1982, the OSR planned and implemented the first phase of an information program aimed at preventing blindness from neovascular senile macular degeneration (SMD). The program is designed to communicate the sight-saving results of an NEI-supported clinical trial which showed that laser photocoagulation dramatically reduces the risk of severe visual loss from the neovascular form of this disease.

Before the study was initiated, some investigators believed that photocoagulation could slow down the rate of degeneration in SMD, but others thought that this laser treatment might be ineffective or even accelerate loss of vision in some patients. After only 3 years of the 5-year study, members of the Data and Safety Monitoring Committee discerned a significant pattern in the treatment results. Sixty percent of the untreated eyes with neovascular SMD lost most of their vision, while only 25 percent of the treated eyes had such a serious outcome.

When this dramatic difference between treated and untreated eyes was observed, the study protocol was changed to allow for immediate treatment of all patients enrolled in the study who might benefit, and plans were made for the orderly release of the findings to appropriate target audiences. A scientific report of the research results was prepared for submission to the Archives of Ophthalmology, and when it was accepted, the NEI distributed a preprint of the

report to every ophthalmologist in the country. This was done to maximize the chances for people who were then in the treatable stage of SMD to be spared visual loss: some people who had SMD and whose eyesight was salvageable might no longer be in a treatable stage of the disease when the scientific report was actually published. The preprint was also sent to medical news publications, vision care and vision research publications, and voluntary and professional organizations with an interest in eye care, vision research, and/or epidemiology.

After the preprints were mailed, the NEI announced the study results to the general public at a news conference held May 6, 1982. The news conference was attended by representatives of every major television network and most DC area stations as well. Broadcast coverage was extensive on the day of the news conference, with Dan Rather, David Shoumacher, and Roger Mudd all addressing the issue; and it continued for many days as stations around the country picked up on the news. A report of the findings also appeared in hundreds of newspapers and magazines across the Nation, including of course the Washington Post, New York Times, Wall Street Journal, and the weekly news publications.

Articles appearing in the print media focused on the dramatic reduction in risk of visual impairment achieved by laser treatment, but most were careful to note that the study dealt with the use of this treatment in only one type of SMD, and that people with this type represented only a small proportion of all those who have the disease. This point was emphasized repeatedly at the news conference and in information materials distributed by the OSR.

It is now time to complete an assessment of the effects of our efforts to date, attempt to clear up any misconceptions, develop a plan for broader dissemination of the results to those who provide primary care, and attempt to get the results properly incorporated into educational materials. Simultaneously, we will continue to strive for broader dissemination of the results to appropriate segments of the general public.

Scientific Reporting and Knowledge Transfer

The communication of clinically applicable research results to appropriate target audiences (see above for two examples) is a primary function of the Office of Scientific Reporting and requires a major commitment of staff time and resources to scientific reporting and knowledge transfer activities. In addition to the projects described above, the Office distributed information about other NEI-supported clinical trials and programs to ophthalmologists, optometrists, epidemiologists, and neuroscientists. The Office responded to inquiries from the medical and scientific press and stimulated press coverage of NEI programs, policies, and research results in appropriate journals and scientific publications. Assistance was provided to various components of the Institute seeking to make their programs and projects known to the scientific community and to enlist the cooperation of scientists.

An exhibit highlighting advances in vision research during the last decade was produced for display at a joint meeting of the International Congress of Ophthalmology and the American Academy of Ophthalmology. In addition, the Office was responsible for planning and implementing an international campaign to disseminate information about prevention of blindness programs of the International Agency for the Prevention of Blindness (IAPB). The goal of IAPB, and of the campaign, was to foster the development of blindness prevention programs throughout

the world. Representatives of local, national, and international news agencies were contacted and were expressing interest in covering the agency and its activities as this Annual Report was going to press.

Consumer Education

The first phase in a major information campaign on diabetic retinopathy and its treatment is well underway (see Diabetic Retinopathy above). Preliminary efforts to obtain both broadcast and print media coverage have been extremely successful, and the experience has been helpful in planning further efforts in this regard. National Eye Institute/private sector collaboration on programs to prevent needless blindness is being expanded. In fact, progress has been made in enlisting the cooperation and assistance of voluntary organizations and professionals to promote and distribute NEI information materials. The Office also intends to work with the Audiovisual Branch of NIH on production of several public service announcements featuring diabetic retinopathy and its treatment.

In addition to providing information on diabetic retinopathy, senile macular degeneration (see above), and many other eye disorders, the OSR staff prepared for publication an information brochure on cataract, its treatment, and research advances in the field. Other booklets on senile macular degeneration and low vision are in draft.

Media Relations

Communication of clinically applicable research results that could help to prevent eye disease and visual impairment is the primary objective of the OSR's media relations program. In addition, the Office provides assistance to reporters seeking objective information about the eye, treatment of eye disease, and vision research. Often, it is possible to prevent inaccurate and misleading statements or articles that, if reported, would adversely affect the eye health of the American people or the research programs of the National Eye Institute.

Public Inquiries

As expected, the volume of public inquiries increases as the Office successfully stimulates press coverage of NEI programs and advances in vision research. However, increasing reliance on use of the telephone to respond to inquiries and the availability of a few new brochures and fact sheets continue to reduce the relative amount of paperwork and OSR's response time for routine inquiries.

Special Requests

Serving in an advisory capacity to the senior staff of the NEI, the Office of Scientific Reporting is frequently consulted about the impact of NEI policies on the general public, scientific community, and Congress. The Office is also responsible for preparing the NEI Director's Opening Statement before the House and Senate Appropriations Committees, editing the transcripts from these hearings, and researching and writing answers to questions asked by committee members. OSR staff prepares the Special Reports to Congress as well and assists in the review and editing of other politically sensitive documents, including the Institute's annual budget statement and the NEI's annual submission to the NIH Diabetes Mellitus Coordinating Committee. The OSR staff members also advise on preparation

of scientific manuscripts, exhibits, and audiovisual materials, distribution of information to the scientific community, and responses to Freedom of Information Act requests. The annual Presidential proclamation for Save Your Vision Week is prepared by OSR, and the staff is also collaborating on the development of printed materials to be used in conjunction with the national Visual Acuity Impairment Survey.

INTRAMURAL RESEARCH



ANNUAL REPORT
NATIONAL EYE INSTITUTE
October 1, 1981 - September 30, 1982

REPORT OF THE SCIENTIFIC DIRECTOR
Jin H. Kinoshita, Ph.D.

The NEI intramural program is one of the most comprehensive vision research enterprises in the world. The establishment of the Laboratory of Molecular and Developmental Biology adds a new dimension to the vision research activities at the Bethesda campus. This Laboratory, the Laboratory of Sensorimotor Research created a few years ago, and the well-established Clinical Branch and Laboratory of Vision Research, include an impressive array of talent allowing for multidisciplinary approaches to problems causing blindness and to understanding the fundamental nature of visual processes.

Because of the dwindling level of financial support, future expansion of the intramural program does not appear realistic. However, we do have in place the necessary components to continue to have an impact on the future development of the vision research field. Even though the NEI intramural program plays an important role in training vision researchers, the existence of the intramural program is mainly justified by its research accomplishments. Anyone perusing this year's project reports cannot be but impressed by the achievements of the research conducted at the NEI. It is quite clear that a number of laboratory research projects are aimed at solving clinical problems. Reports reveal that some of the research has progressed to a stage where promising laboratory leads to the correction of eye disorders are being tested in the clinic. These studies exemplify the emphasis placed in our program of bridging the gap between the laboratory and clinic so that clinical problems can be examined, analyzed, and possibly treated by new and sophisticated methods developed in the laboratory.

The utilization of the Ambulatory Care Research Facility, which is now occurring, will enable a greater degree of collaboration among various components of the NEI. Therefore, even though additional resources may not be forthcoming, the consolidation of activities and the greater cooperation among existing components should yield even more exciting accomplishments in the future.

Clinical Branch



ANNUAL REPORT
NATIONAL EYE INSTITUTE
October 1, 1980 - September 30, 1981

REPORT OF THE CLINICAL DIRECTOR
Elmer J. Ballantine, M.D.

The Clinical Branch is organized into six sections, each with a section head: Ophthalmic Immunology, Dr. Robert Nussenblatt; Glaucoma, Dr. Douglas Gaasterland; Neuro-ophthalmology, Dr. David Cogan; Clinical Eye Pathology, Dr. Merlyn Rodrigues. Two new sections were established in the past year; Ophthalmic Genetics and Pediatric Ophthalmology, Dr. Muriel Kaiser-Kupfer and Visual Processing, Dr. Francisco deMonasterio. Twenty-eight clinical research protocols are active.

Development of a Q-switched ruby laser and a Q-switched neodymium argon laser for use in the anterior segment of the eye has progressed so that a Q-switched ruby laser is suitable for use in patients and a protocol for opening pupillary membranes is underway.

Development of Q-switched lasers with a spot size of 25 microns for use in trabeculoplasty is proceeding. As soon as experience with this laser in treating trabecular meshwork of monkeys has proved it to be safe for human use, we will begin a randomized clinical trial comparing Q-switched to argon laser trabeculoplasty.

A carbon dioxide laser has been adapted for cutting vitreous membranes during vitrectomy. A method for inducing membranes in the vitreous of rabbits and monkeys was developed. The feasibility of cutting membranes with the CO₂ laser has been demonstrated in these eyes. Because the 10.6 radiation of the CO₂ laser does not penetrate water and is absorbed at the surface of water containing tissues, it is likely that probes can be developed that will permit cutting of vitreous membranes close to the retina without injuring it.

Five patients have been recruited for the protocol for treating severe, posterior uveitis with Cyclosporin A. A protocol for treating ocular toxoplasmosis by randomly assigning patients to receive either clindamycin and sulfadiazine or daraprim and sulfadiazine. If clindamycin is shown to be as effective as daraprim, the patients will not have to risk the serious side effects of daraprim.

A continuing study of HLA, ABO, and B-cell alloantigens in patients with a variety of ocular inflammatory disease has shown that 80% of the patients with "Birdshot retinochoroidopathy" were positive for the antigen HLA - A29. The computed relative risk indicates that persons with this antigen are 50 times more likely to have the disease than those who lack the antigen. This is one of the highest relative risks ever reported.

Experiments to investigate the mechanisms of the experimental allergic uveitis (EAU) induced in animals by immunizing with "S-antigen" in complete Freund's adjuvant were continued. It was shown the EAU could not be induced in

homozygous athymic nude rats. When EAU was induced in heterozygous nude rats and T cells from these rats were transferred to the homozygous rats, ocular inflammatory disease occurred in five to seven days. These studies support the idea that the EAU is T cell dependent.

The lymphocytes of some patients with ocular inflammatory disease (uveitis) showed a positive "memory" response to S antigen in that they underwent blast transformation when exposed to "S" antigen in vitro while lymphocytes from normal subjects did not transform. Cells from some of these patients also had abnormalities of suppressor cell functions. This is the first demonstration of lymphocyte immune "memory" to a purified retinal antigen.

The clinical trial of a low arginine, low protein diet to prevent the visual loss in patients having hyper-ornithinemia and gyrate atrophy of the retina and choroid was continued.

Two patients maintained on the diet, for 41 and 15 months had an improvement in dark adaptation, averaged ERG and color vision. Twelve patients have been entered in this protocol. Compliance with the diet remains a major difficulty.

Progressive essential iris atrophy is a rare disease usually affecting one eye only of young adults. Five times as many women as men are affected. The even rarer bilateral cases are almost exclusively in men. Only a few cases could be familial. Atrophy of the iris proceeds inexorably, eventually glaucoma develops that is intractable to treatment and the affected eye becomes blind. The cause is completely unknown and there is no effective treatment.

A group of six patients with this condition is being studied in an effort to discover the pathogenic mechanisms and to develop effective treatment. Although most cases are superficially unilateral, careful study of the opposite eye has shown that it may have subclinical involvement with decreased outflow facility, iris transillumination or corneal endothelial abnormalities. These observations suggest that the causes cannot be purely local in the affected eye.

A new syndrome in which the homosexual males have an acquired immuno deficiency results in devastating infections by viruses and microorganisms that in normals produce little or no permanent injury.

In many of these patients there is a severe retinopathy which may destroy useful vision. In one case in which the lesions were carefully observed prior to death, the eyes were obtained at autopsy. The virus of cytomegalic inclusions disease was shown to be the cause of the retinal disease.

A new syndrome was described in which patients with a deficiency of sphingomyelinase have splenomegaly and a unique ring of crystalloid retinal opacities surrounding the foveola. The syndrome is probably a benign form of Niemann-Pick disease and has been called the "Macula Halo Syndrome".

Improvements in the detailed recording of eye movements were completed by adapting the search coil technique for use in patients. In this procedure two mutually perpendicular sets of field coils carrying alternating currents of different frequencies surround the patients head and induce potentials in a

search coil embedded in an annular contact lens on the patients eye. The induced potentials vary with the position of the coil with respect to the field coils. Computer processing of these signals produces a precise record of the dynamics of eye movements free of blink and head movement distortions.

These methods have been used to study the relationship of cerebellar function to adaptation to a sixth nerve palsy, blink-saccade synkinesis, downbeat nystagmus, and periodic alternating nystagmus in patients having the Arnold-Chiari malformation.

Study of the mechanism by which Procion yellow and its congeners stain blue cones in monkey retinas indicates that the blue cones stain because they are more susceptible to injury than all the other cones. These observations offer a partial explanation for Kllner's rule that in retinal disease, blue-yellow discrimination is impaired while in optic nerve disease, red-green discrimination is impaired.

Electroretinographic study of human subjects of various ages using color stimuli to accentuate the B-wave responses of dark adapted rods, blue cones and red-green cones showed that with advancing age all responses are reduced but, after correction for the yellowing of aged lenses, blue cone responses are preferentially reduced.

Blue cone staining of the retinas from young and aged monkeys confirms that the number of blue cones decreases with age and to a greater degree in the parafoveal region.

Apparatus is being developed for determining the loci of neutral points, (i.e. those colors that are confused with achromatic light) in patients with acquired color vision defects. Changes in these loci may be characteristic of various retinal diseases and may be sensitive indicators of the stage of the disease and its recovery.

An automatic "forced choice procedure" for testing spatial contrast sensitivity was developed for clinical use. An unexpected finding was that when the central area of the visual field is blocked, the pattern of spatial frequency loss depends on whether the contrast was varied gradually or abruptly.

These procedures have been used in a study to determine the pattern of loss and recovery of visual functions associated with chiasmal and retrochiasmal lesions producing hemianopic visual field defects. The temporal course of color vision defects and spatial contrast sensitivity were correlated with static and kinetic perimetry and neuroradiologic estimation of the size and location of the lesions. Contrast sensitivity and color vision were impaired if perimetric defects were present but they did not occur in the absence of visual field defects. Recovery of the impaired visual field was accompanied by improvement in contrast sensitivity and color vision but their improvement lagged the improvement in visual fields. These two functions are being developed as sensitive tests of residual damage and its recovery after visual fields have become normal.

Radio-labelled 2-deoxyglucose (2DG) is taken up by cerebral neurons in intact monkeys in amounts that depend on the physiologic activity of the cells.

Intracellular it is phosphorylated to 2DG-6 phosphate but is not further metabolized and remains in the cells.

A method using this reaction has been developed for localizing neural activity in the brain. The frozen brains containing the intracellular 2DG-6-phosphate are subjected to solvent exchange for water in the frozen state and subsequent embedding, sectioning and development of radioautographs. There are no significant losses or migration of the radiolabels and 20 features can be resolved. By using this method, discrete regions of the visual cortex that become active during a period of a specific visual stimulus can be defined.

Investigation of the functions of area of V4 of the extrastriate visual cortex of monkeys was continued using extracellular recording with pattern stimuli of various colors. The response to pattern stimuli was affected by color in half of the responding cells. Stimuli in large surrounding areas of the retina were unable to produce responses in these striate cortex cells but did alter the effect of color on the response to pattern stimuli. These very large "silent" surrounds are a new feature in image processing by the brain.

The orderly arrangement and interaction of the extracellular macromolecules and the enzymatic processes responsible for their metabolism are important factors in determining permeability of the trabecular meshwork to aqueous humor and the transfer of substances through Bruch's membrane. Methods have been developed for studying these processes by de-aggregating and extracting the intact glycoproteins and proteoglycans from the tissues, their separation and characterization by chromatographic methods, immuno-precipitation gel electrophoresis, density centrifugation and their digestion by specific enzymes.

In isolated monkey trabecular meshwork, more hyaluronic acid was synthesized relative to the rate of synthesis of glycoproteins and proteoglycans, while in the *insitu* meshwork the amounts synthesized were reversed.

Using these methods it was shown that newly synthesized proteoglycans containing 65% chondroitin-dermatin sulfate and 35% heparan sulfate with molecular weights between 100 and 150 kilodaltons were incorporated into Bruch's membrane in organ culture. Glycosaminoglycan side chains had a molecular weight of 44 kilodaltons.

The working out of these details is a necessary preliminary to the study of the alteration of the trabecular meshwork by intraocular pressure raising drugs and in surgical specimens from human glaucoma patients and in Bruch's membrane from human eye bank eyes.

A study of the distribution and characteristics of Langerhan's cells (LCs) in human corneal and conjunctival epithelial sheets using histochemical, immunofluorescence and immunoelectron microscopic methods was completed. The LCs stained positive for ATPase and with antibodies to HLA-DR antigen and were negative for DOPA-oxidase. In human conjunctiva there were 250 to 300 LCs/mm² compared to 15 to 20/mm² in the peripheral third of the corneal epithelium.

Specimens of trabecular meshwork were obtained from patients having uncontrolled open angle glaucoma when trabeculectomy was performed three hours to one year after argon laser trabeculoplasty.

Scanning and transmission electron microscopy of these specimens provided detailed record of the development of laser lesion in human trabecular meshwork. The laser-treated sites showed irregular areas of disruption and obliterated trabecular beams. In more recent burns, fibrinous material and occasional macrophages were present. Specimens examined after a longer interval following laser showed considerable fibrosis at the treated sites. The laser burns primarily involved superficial and midtrabecular meshwork and did not extend to Schlemm's canal.

In five trabeculectomy specimens from eyes in which laser trabeculectomy or iridotomy was followed by persistent elevations of intraocular pressure. The meshwork contained macrophages, lymphocytes activated trabecular endothelial cells and tissue debris from trabecular beams. This inflammatory response appears to be an important factor in the acute post laser elevation of intraocular pressure.

Investigations on the origin of the cells in periretinal membranes continues. Periretinal membranes obtained by vitrectomy from three patients with massive periretinal proliferation had abundant glial cells. The membranes contained glial fibrillary acidic protein but did not contain Collagen types I, II and IV.

In trabeculectomy specimens from cases of Chandler's syndrome, it was shown that the abnormal corneal epithelium had grown across the inner uveal meshwork. This could be the cause of the glaucoma in this syndrome.

Specimens of cornea obtained at keratoplasty from patients having posterior polymorphous dystrophy. The posterior surface of the cornea was covered by a mixture of endothelial epithelial cells and tissue culture of this cell layer grew out the same mixture of cell types. Epithelial cells were shown to stain positively with antibody to human epidermal keratan while the endothelial cells were unstained.

This reaction has been shown to distinguish these corneas from those of Chandler's syndrome, in which none of the atypical corneal endothelial cells stain for epidermal keratan.

A new syndrome - polymorphic amyloid degeneration of the cornea, was described in 14 patients and differentiated clinically and histologically from lattice corneal dystrophy, another amyloid deposition disease.

Twenty-seven of forty-four patients with Gaucher's disease had pingueculae. None of the pingueculae had Gaucher cells in them. This contradicts previous reports of smaller series by others.

Work continues with several animal models of human eye disease. A strain of the encephalomyocarditis virus infecting mice provided hypoglycemia and glycosuria. There were no ophthalmoscopically apparent lesions and the only ocular abnormality was decreased numbers of pericytes in trypsin digests of the retinas. The kidneys of the affected mice had nodular and diffuse glomerulosclerosis and mesongial thickening similar to human Kimmelstiel-Wilson diseases. This model may yet be useful in the study of diabetic retinal vascular disease.

During the year the Section on Clinical Eye Pathology processed fifty autopsy eyes. One-hundred and seventy eyes were obtained from the Eye Bank and the tissues distributed to investigators in the Clinical Branch and the Laboratory for Vision Research. Fifty-five biopsies and surgical specimens were processed.

Three-hundred and twenty animal eyes and other tissues were processed for investigations related to diabetes, uveitis, and retinal degeneration. Two-hundred and fifty tissue specimens were processed for transmission electronmicroscopy. Two-hundred and forty specimens were processed for scanning electronmicroscopy.

There were 4,550 outpatient and inpatient visits referred from other Institutes to the NEI clinical facilities. Ninety-eight inpatients were admitted and 17 surgical operations were performed. The Clinical Branch continued to cooperate with other NIH Institutes in pursuit of timely research opportunities. A cooperative study of breast cancer patients continues in cooperation with the National Cancer Institute, the study of diabetic retinopathy in the Pima Indian was continued with the Southwestern Fields Studies Section of NIAMDD.

A study of the ocular complications in a group of sixty patients having the hypereosinophilic syndrome is underway with investigators in NIAMDD. The Neuro-ophthalmology Section continues to cooperate in the study of the ocular lesions of patients in the NINCDS Developmental and Metabolic Neurology Branch. The Clinical Branch provides a pediatric ophthalmology representative to the Inter Institute Genetics group. Clinical Branch scientists continue to serve as consultants to the National Institutes on Drug Abuse, Interagency Committee on New Therapies for Pain and Discomfort, and the International Vitamin A Consultative Group.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00150-09 CB
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PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Ocular Hypertension Study

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Elmer J. Ballantine	M.D.	Clinical Director	CB	NEI
Other: Douglas E. Gaasterland	M.D.	Chief, Section on Glaucoma	CB	NEI
		Richard Weiblinger B.S.	CB	NEI
		Biologist		

COOPERATING UNITS (if any)

Office of Biometry and Epidemiology, NEI

LAB/BRANCH

Clinical Branch

SECTION

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.6	0.4

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Patients with ocular hypertension are randomly assigned to treatment with topical pilocarpine in one or both eyes or to no treatment. The objectives of the study are: 1) to determine if treatment with pilocarpine to reduce intraocular pressure before visual field changes occur will reduce the number of ocular hypertensive subjects who eventually become glaucomatous, and 2) to determine if measurements of aqueous humor dynamics, the response to water loading of diurnal variation in intraocular pressure, serial stereophotographs of the optic disc, and measurements of visual fields help to predict which patients will eventually become glaucomatous.

Project Description:

Protocol Number: 77 EI 38

Objectives: Prolonged observation of a series of patients with ocular hypertension, some of whom are treated with miotics, will help to determine which signs have value in predicting those who will eventually require treatment and to determine if early treatment of ocular hypertension has any value in preventing visual field loss or in slowing the rate of development of abnormalities of aqueous humor dynamics.

Methods Employed: A detailed plan for classifying patients with ocular hypertension; observing them by repeated examinations including measurement of visual fields, aqueous humor dynamics, and photogrammetry of the optic disc over a period of five or more years; and randomly assigning patients to treatment with pilocarpine collyria in one or both eyes, or to no treatment, has been standardized.

Major Findings: There has been no indication that the course of ocular hypertension has been affected by treatment.

Although visual field losses are said to be preceded by glaucomatous changes in the optic discs, in this study we have documented the development of an undoubted glaucomatous visual field loss in the absence of any change in optic disc appearance.

One-hundred thirty patients have been examined to determine eligibility, and 38 are under continuing observation following randomization to treatment groups.

Significance to Biomedical Research and the Program of the Institute: Early, precise identification of patients who require treatment because they are in the early stages of simple glaucoma remains an unsolved problem. The data being collected in this study will furnish a basis for establishing criteria for treatment more precisely than is now possible. There is at present little detailed knowledge of the progression of optic disc changes in ocular hypertension. The data being collected in this study, as well as the development of better instruments for the measurements in this study, will supply needed information in this field.

Proposed Course: We expect that the project will continue for at least five years, and that 100 subjects will be randomized to the treatment groups.

NEI Research Program: Glaucoma--Primary Open-Angle Glaucoma

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00022-08 CB

PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Urokinase Central Retinal Vein Occlusion Trial

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Elmer J. Ballantine	M.D.	Clinical Director	CB	NEI
Other:	Harvey R. Gralnick	M.D.	Chief, Hematology Service	CC	NIH
	Daniel G. Seigel	Sc.D.	Deputy Chief, Office of Biometry	OBE	NEI

COOPERATING UNITS (if any)

Office of Biometry and Epidemiology, NEI

LAB/BRANCH

Clinical Branch

SECTION

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

0.1

PROFESSIONAL:

0.1

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS

(b) HUMAN TISSUES

(c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Patients with recent complete occlusion of the central retinal vein are randomly assigned to treatment either with intravenous urokinase followed by heparin, heparin alone or intravenous fluids alone. The patients are then examined periodically for one year, and the effectiveness of treatment is judged by restoration of vision and the degree of protection achieved against the development of hemorrhagic glaucoma.

Project Description:

Protocol Number: 75 EI 100

Objectives: To determine if treatment with thrombolytic agent (urokinase) plus anticoagulation with heparin, or treatment by anticoagulation with heparin alone, is effective in reducing the loss of visual acuity and the progression to hemorrhagic glaucoma that is a consequence of occlusion of the central retinal vein.

Methods Employed: Patients are examined according to a detailed plan to determine eligibility for the study. Eligible patients, if they agree to participate, are assigned by randomization to one of three treatment plans:

(1) Twenty-four hours of continuous intravenous treatment with urokinase in an effort to resolve the occlusion of the central retinal vein. This is followed by two weeks of anticoagulation treatment with heparin to prevent reformation of venous obstruction.

(2) Heparin anticoagulation alone.

(3) Hospitalization and administration of intravenous fluids similar in volume to those used in the other treatment groups.

After the treatment period, the patients are examined periodically for one year to determine the rate at which hemorrhagic glaucoma occurs and the degree of restoration of vision to the eye. After one year they are examined at yearly intervals.

Major Findings: Twenty patients have been examined to determine their eligibility and seven patients have been randomized to treatment. No trends have been observed.

Significance to Biomedical Research and the Program of the Institute: Occlusion of the central retinal vein is a serious cause of visual disability, and one of its major consequences is hemorrhagic glaucoma, which almost invariably results in a blind, painful eye. In the past, treatment with anticoagulation has been advocated, but no convincing evidence of effectiveness has been published. With the development of an effective thrombolytic agent (urokinase), the possibility of dissolving the presumed cause of the obstruction, a thrombus in the central retinal vein, and the demonstration that urokinase is effective in thrombolytic disease in other sites support the decision to undertake this trial.

Proposed Course: Examination of published data on the course of occlusion of central retinal vein indicates that 75 patients will need to be recruited to demonstrate that a 50 percent improvement in vision is produced by the treatment. Recruitment has been slow, mainly because the present protocol requires two weeks hospitalization for each patient. The protocol is now being revised to shorten the period of hospitalization. We will continue to recruit until 75 patients have been treated.

Project No. Z01 EY 00022-08 CB

NEI Research Program: Retinal and Choroidal Diseases--Diabetic Retinopathy
Sickle Cell Retinopathy, and Other Vascular Abnormalities

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
**NOTICE OF
INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 EY 00118-02 CB

PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Septic Chorioretinitis in Animals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Sanford M. Meyers M.D. Senior Staff Ophthalmologist CB NEI
Other: Merlyn M. Rodrigues M.D. Chief, Section on Clinical CB NEI
Eye Pathology

COOPERATING UNITS (if any)

Section on Clinical Eye Pathology, NEI

LAB/BRANCH

Clinical Branch

SECTION

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.35	0.25	0.10

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Multifocal choroiditis with overlying retinal detachment occurs after carotid injection of certain bacteria in dogs. The ocular lesions occur mainly in the tapetal area of the retina, correlate with microabscesses in the inner choroid and subretinal space, and occasionally occur in the inner retina and anterior uveal tract. The major pathophysiologic factor involved in the dog model of septic choroiditis appears to be embolization of the choriocapillaries by "live" bacteria which clump and adhere well to tissues. In the dosages used, antibiotics did not prevent or alter the severity of the fundus lesions. In pigtail monkeys carotid injection of a dextran producing strain of Streptococcus mutans consistently caused fundus lesions clinically resembling those seen in humans with bacteremia. In contrast to the dog model the lesions in the monkey occurred mainly in the retina but also in the choroid and optic nerve.

Project Description:

Objectives: This study was designed to correlate the clinical and pathologic features in the dog model of septic chorioretinitis, to investigate the pathophysiologic mechanisms involved in this model, and to determine if similar fundus lesions occur in pigtail monkeys.

Methods Employed: Mongrel dogs had carotid injection of certain bacteria. In pigtail monkeys (Macaque Nemestrina) only a dextran producing strain of Streptococcus mutans was used because it consistently caused fundus lesions with only minimal systemic effects. Fundus lesions were documented with fundus photography and correlated with the histopathologic findings. To investigate the pathophysiologic mechanisms, various bacteria, the effect of antibiotics, and altering dextran production of a dextran producing strain of Streptococcus mutans were studied in the dog model.

Major Findings: Carotid injection of certain bacteria consistently cause fundus lesions in dogs and pigtail monkeys. The major pathophysiologic mechanism appears to be embolization of choroidal and retinal vessels by "live" bacteria, which clump and adhere well to tissues. In the dosages used antibiotics did not prevent or alter the severity of the fundus lesions.

Significance to Biomedical Research and the Program of the Institute: The chorioretinal lesions observed in this animal model resemble the fundus lesions which have been described in human cases of bacteremia unassociated with diabetes mellitus, blood dyscrasias, hypertension, or collagen vascular diseases. The data in this study support the hypothesis that the fundus lesions observed in human cases of bacteremia result from embolization by "live" bacteria. Thus, it appears that a detailed fundus examination can be helpful in assessing the extent of a systemic infection and new fundus lesions may signify recurrent infection or incomplete treatment. Additionally, fundus lesions, at times, may be the initial sign of a systemic infection. It is possible that some cases of idiopathic choroiditis, like central serous choroidopathy, may be associated with subclinical episodes of bacteremia.

Proposed Course: This project will continue for the next year to observe the long term course of the fundus lesions in one of the pigtail monkeys.

NEI Research Program: Retinal and Choroidal Diseases--Inflammatory Disorders

Publications:

Meyers SM, Vasil ML, Yamamoto L: Pathophysiology of multifocal choroiditis with retinal detachment after carotid injection of Streptococcus mutans and other bacteria in dogs. Invest Ophthalmol Vis Sci 22:165-173, 1982.

Meyers SM, Rodrigues M, Vasil ML: Fundus lesions after carotid injection of Streptococcus mutans in monkeys. Infect Immun: 35:1079-1085, 1982.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00119-02 CB

PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Fibronectin Concentration in Eyes with Membranes Undergoing Vitrealretinal
Surgery

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER
PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Sanford M. Meyers	M.D.	Senior Staff Ophthalmologist	CB	NEI
Other:	John Hassell	Ph.D.	Research Biologist	LDBA	NIDR
	Robert Nussenblatt	M.D.	Chief, Section of Ophthalmic Immunology	CB	NEI

COOPERATING UNITS (if any)

LAB/BRANCH
Clinical Branch

SECTION

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.45	0.25	0.20

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study will determine if the vitreous concentration of fibronectin in cases with vitreal or periretinal membranes differs from that in normal autopsy human eyes. Vitreous samples taken during vitrectomy from cases of decreased vision due to periretinal or vitreal membranes will be assayed for fibronectin concentration and processed for histopathologic examination. The findings will be correlated with the types of ocular disease causing the periretinal or vitreal membranes. In uveitis or retinal vasculitis cases in which vitreous surgery is indicated, the vitreous specimens will be processed for immunologic testing.

Project Description:

Protocol Number: 81-EI-28

Objectives: This study is designed to determine if the vitreous concentration of fibronectin in eyes with vitreal membranes differs from that in autopsy human eyes, to correlate these findings with clinical features of the membranes, and to perform immunologic studies on those vitreous specimens or from cases of uveitis or retinal vasculitis undergoing vitrectomy for dense vitreous opacities.

Methods Employed: Patients with decreased vision because of vitreal membranes will be recruited for vitrectomy surgery. The patients will have complete ocular examinations with detailed examination of the vitreous and retina. Vitreous samples taken at surgery and pre and post operative plasma samples will be immediately frozen. The fibronectin concentration will be measured using an enzyme-linked immunosorbent assay which is being developed.

In cases of retinal vasculitis or uveitis undergoing vitrectomy, immunological tests will be performed on the vitreous specimens removed during vitrectomy.

Major Findings: Data on this project will be collected over the next three years. The combined illumination-irrigation 20-gauge probes and the modified scleral plugs, developed in collaboration with the Division of Research Services Biomedical Engineering Instrumentation Branch, have performed well in vitrectomy cases, in humans, and are currently being used in all vitrectomy cases at the National Eye Institute.

Significance to Biomedical Research and the Program of the Institute: The data from this study may enhance our understanding of the pathophysiologic mechanisms involved in uveitis and vitreal membrane formation. Vitreous and periretinal membrane formation is the major cause of failure after retinal or vitreous surgery. The combined illumination-irrigation 20-gauge probes and modified scleral plugs have improved the currently available instrumentation for vitreous surgery.

Proposed Course: The project will continue for the next three years.

NEI Research Program: Retinal and Choroidal Diseases--Diabetic Retinopathy, Sickle Cell Retinopathy, and Other Vascular Abnormalities/ Retinal Detachment and Vitreous Disorders

Publications:

Meyers SM, Gaasterland DE: Scleral plugs with an attached suture for use during vitrectomy. Am J Ophthalmol 92:744, 1981.

Meyers SM, Bonner RF, Leighton SB: Combined illumination-irrigation 20-gauge probes for vitrectomy. Arch Ophthalmol 100:622-623, 1982.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00120-02 CB

PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Laser Instrumentation for Vitreous Surgery

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Sanford M. Meyers	M.D.	Senior Staff Ophthalmologist	CB	NEI
Other:	Robert F. Bonner	Ph.D.	Physicist	BEIB	DRS
	Stephen B. Leighton	Ph.D.	Engineer	BEIB	DRS
	Merlyn M. Rodrigues	M.D.	Chief, Section on Clinical Eye Pathology	CB	NEI

COOPERATING UNITS (if any)

Division of Research Services Biomedical Engineering Instrumentation Branch

LAB/BRANCH

Clinical Branch

SECTION

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

0.85

PROFESSIONAL:

0.60

OTHER:

0.25

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS

(b) HUMAN TISSUES

(c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A carbon dioxide laser with a special delivery system for use in vitreous surgery has been developed and is undergoing testing in animals. Preliminary data reveal that the carbon dioxide laser instrument appears beneficial in certain aspects of vitreous surgery.

Project Description:

Objectives: This study is designed to develop and test the efficacy of a carbon dioxide laser instrument for use in vitreous surgery.

Methods Employed: A carbon dioxide laser with a special delivery system adapted for use in vitreous surgery is being developed. The safety and efficacy of the prototype unit is being tested in rabbits and monkeys with vitreal membranes. The vitreal membranes in these animals are created using standardly accepted methods. The findings are documented with photography. The animal eyes are examined clinically and pathologically at selected times after surgery.

Major Findings: Preliminary data reveals that the carbon dioxide laser can cut experimentally created membranes in rabbits. During the development of this carbon dioxide laser instrument, combined illumination-irrigation 20-gauge probes for vitrectomy in humans were built. These vitrectomy instruments performed well in human cases and are currently being used in all human vitrectomy cases at the National Eye Institute.

The rabbit model for vitreal membranes used to test the carbon dioxide laser was also used to determine the effect of selected antimitotic drugs on vitreal membrane formation. Intravitreal injection of cytosine arabinoside, doxorubicin, or dexamethasone did not alter the frequency or severity of the resultant vitreal membranes.

Significance to Biomedical Research and the Program of the Institute: Although the present mechanical vitrectomy instruments perform well in most cases, there is a risk of intraoperative complications (retinal tears and hemorrhage) when vitreal membranes are cut, especially if the membranes are taut and have a strong adhesion to the retina. Tension on the membranes is increased as the cutter of the vitrectomy instrument or the vitreous scissors cuts the tissue. This tension is transmitted to the vitreoretinal adhesion and surrounding retina predisposing this area to hemorrhage and retinal tears. If the vitreal membrane is vascularized, this tension may cause bleeding. The carbon dioxide laser vitrectomy may decrease the incidence of these intraoperative complications and increase the facility in cutting selected vitreal membranes.

The combined illumination-irrigating 20-gauge probes have improved the currently available instrumentation available for vitreous surgery.

Proposed Course: If further investigation of the prototype carbon dioxide laser in animals documents the efficacy and safety of the instrument in vitreous surgery, a clinical trial in selected patients undergoing vitreous surgery will be initiated.

NEI Research Program: Retinal and Choroidal Diseases--Diabetic Retinopathy, Sickle Cell Retinopathy, and Other Vascular Abnormalities/ Retinal Detachment and Vitreous Disorders

Publications:

Meyers SM, Bonner RF, Leighton SB: Combined illumination-irrigation 20-gauge probes for vitrectomy. Arch Ophthalmol 100:622-623, 1982.

Meyers SM, Rodrigues M: Effect of selected intravitreal drugs after severe penetrating injury in rabbits. Curr Eye Res 1:471-477, 1981.

PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Histopathology of Human Corneal Dystrophies and Degenerations

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Merlyn M. Rodrigues	M.D.	Chief, Section on Clinical Eye Pathology	CB	NEI
Other:	Joseph Hackett	B.S.	Biologist	CB	NEI
	Gunter Thomas		Biologist	CB	NEI

COOPERATING UNITS (if any)

Department of Ophthalmology, University of Iowa

LAB/BRANCH
Clinical BranchSECTION
Section on Clinical Eye Pathology

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.3	0.1

CHECK APPROPRIATE BOX(ES)

 (a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER (a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Human corneal dystrophies and degenerations, which have been clinically documented are studied as keratoplasty specimens with histochemical stains, scanning and transmission electron microscopy, and immunologic techniques in an attempt to elucidate pathogenetic mechanisms. This approach has provided insight into cell-to-cell relationships in the normal and diseased states. In patients with primary and recurrent macular corneal dystrophy, intercellular and extracellular accumulation of fibrillar granular material was observed in the corneal stroma, Descemet's membrane and corneal endothelium. The presence and production of collagen, glycoconjugates, and collagenase have been investigated with immunofluorescent, electrophoretic, and chromatographic methods. Electron microscopic studies were performed on keratoconus and pellucid degeneration. Clinicopathologic studies were performed on primary amyloid corneal degeneration.

Project Description:

Objectives: The study attempts to combine detailed clinical and genetic studies of patients with human and corneal diseases, particularly corneal dystrophies, in order to obtain further insight into the mechanisms of corneal opacification.

Methods Employed: Corneal specimens from transplant patients are divided into portions and used separately for light, scanning and transmission electron microscopy. These data provide insight into the morphological appearance of the cells and extracellular materials of the corneal layers. Other portions of the surgical specimens are placed into tissue and cell culture to allow examination of the morphology and biosynthetic activities of the cells of the three corneal layers. Indirect immunofluorescence has shown the range of collagen types present in normal and abnormal tissue. Column chromatography and electrophoresis provide information about the collagen and glycoconjugate biosynthetic patterns of abnormal tissue.

Major Findings:

A. Corneal Dystrophies: Five corneal buttons from patients with hereditary posterior polymorphous dystrophy had an admixture of epithelial-like and endothelial cells on the posterior surface of Descemet's membrane which is normally lined by a monolayer of endothelial cells. The epithelial-like cells were characterized by numerous microvillus projections, prominent desmosomal cell junctions, intracytoplasmic filaments, and scant mitochondria. The adjacent endothelial cells displayed gap junctional complexes, numerous mitochondria with horizontal disposition of cristae, and prominent Golgi. Cells cultured from the corneal endothelium exhibited a similar admixture of cells with epithelial-like and endothelial characteristics. The epithelial-like cells stained positive with antibody to human epidermal keratin, while the endothelial cells were unstained. Corneal tissue with lattice dystrophy stained positive for amyloid with Congo red and displayed dichroism. Immunofluorescence and biochemical studies are in progress to characterize the type of amyloid present. In corneal buttons from patients with macular corneal dystrophy, examination revealed abnormal accumulation of glycosaminoglycan in the corneal stroma as well as in Descemet's membrane and corneal endothelium. The deposits were composed of fibrillar granular material and stained positive with stains for glycosaminoglycans.

B. Corneal Degenerations: i) Keratoconus specimens had the same range of collagen types as normal cornea, with predominantly type I collagen. Type III collagen was detected only in scarred areas. Radioactive labeling experiments on cultures cells from these corneas have demonstrated an elevated production of collagenase compared with the normal. ii) Pellucid corneal degeneration showed thinned corneas inferiorly with no evidence of vascularization. Light and electron microscopy of a corneal button from each patient revealed irregularity of the epithelium in the peripheral thinned areas with a normal Bowman's layer in one case and focal dehiscences in the other. Marked thinning of the corneal stroma accompanied by the presence of a small number of histiocytes was present peripherally in both cases. Descemet's membrane and endothelium were normal. Stromal collagen was normal in diameter and periodicity. In one case,

CM-cellulose and SDS gel profile of the collagens synthesized by these stromocytes in vitro (³H proline label) was similar to those of control corneas and keratoconus. Pellucid corneal degeneration may represent a peripheral form of keratoconus. iii) Polymorphic amyloid degeneration. The occurrence of polymorphic punctate and filamentous opacities in the axial cornea of patients in the fourth decade of life and older is a distinct clinical entity. The glass-like deposits are usually in the deeper layers of the cornea and are associated with normal intervening stroma. Although it is not a cause of visual dysfunction, this disorder may be confused with lattice corneal dystrophy or with the corneal deposits in some dysproteinemias. Common clinical findings were found in 14 patients, and these findings were contrasted with the findings in lattice corneal dystrophy. Family studies failed to demonstrate heritability and lesions were found in older patients only. Histopathologic examination identified the lesions as amyloid. The findings suggest that this disorder should be classified as a corneal degeneration. "Polymorphic amyloid degeneration" is a descriptive term for this condition.

Significance to Biomedical Research and the Program of the Institute:

The mechanisms of opacification and destruction of the cornea in a variety of human diseases must be understood for the improved diagnosis and classification of these entities. This may also lead to a more rational basis for the appropriate treatment of these visually disabling processes. A thorough knowledge of the genetic components of these disorders, if any, will aid in more effective and complete genetic counseling.

Proposed Course: Patient material will be entered into this combined study as it becomes available. Emphasis will be placed on elucidating pathogenic mechanisms in hereditary posterior polymorphous dystrophy, keratoconus, lattice and granular dystrophies. The use of immunological techniques will be expanded to a wider variety of specimens.

NEI Research Program: Corneal Diseases: Corneal Dystrophies, Inherited Disorders and Developmental Anomalies.

Publications:

Rodrigues M, Waring G: Anterior and posterior corneal dystrophies, in Klintworth G, Garner A (eds): Pathobiology of Ocular Diseases. New York, Marcell Dekker Company, 1982, pp. 115-1165.

Rodrigues M, Newsome D, Krachmer J, Sun T-T: Posterior polymorphous dystrophy: Cell culture studies. Exp Eye Res 33:535, 1981.

Rodrigues M, Newsome D, Krachmer J, Eiferman R: Pellucid marginal corneal degeneration. Exp Eye Res 33:277, 1981.

Dubord P, Rodrigues M, Krachmer J: Lattice corneal dystrophy associated with elastosis. Ophthalmology 88:1239, 1981.

Eiferman R, Rodrigues M: Squamous epithelial cysts of the iris. Ophthalmology 88:1281, 1981.

Project No. Z01 EY 00078-05 CB

Mannis MJ, Krachmer JH, Rodrigues MM, Pardos GJ: Polymorphic amyloid degeneration of the cornea. Arch Ophthalmol 99:1217, 1981.

Rodrigues MM, Sun T-T, Krachmer JH, Newsome DA: Posterior polymorphous dystrophy: recent developments. Proceedings of the International Symposium on Genetics in Ophthalmology, New York. Alan Liss, Inc. (in press).

PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Clinicopathologic Studies of Human Ocular Diseases

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Merlyn M. Rodrigues	M.D.	Chief, Section on Clinical Eye Pathology	CB	NEI
Other:	Joseph Hackett	B.S.	Biologist	CB	NEI
	Reginald Gaskins		Histologist	CB	NEI
	Nicole Newman		Histologist	CB	NEI
	Gunter Thomas		Biologist	CB	NEI

COOPERATING UNITS (if any)

Wills Eye Hospital, Philadelphia

Department of Ophthalmology, University of Louisville, Louisville, Kentucky

LAB/BRANCH

Clinical Branch

SECTION

Clinical Eye Pathology

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.2	0.2

CHECK APPROPRIATE BOX(ES)

 (a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER (a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Patients with localized ocular diseases or with ocular manifestations of systemic disease are examined clinically, and photographic documentation is made of significant findings. Biopsy specimens or autopsy eyes from these patients are examined by scanning and transmission electron microscopy and histochemical stains. Studies are performed on patients with glaucoma, ocular and adnexal tumors, vitreoretinal membranes, ocular manifestations of systemic diseases, and laser-induced ocular lesions. Histological studies are also performed on normal human rhesus monkey cornea, iris, and trabecular meshwork and include scanning and transmission microscopy of tissue specimens as well as of cell cultures.

Project Description:

Objectives: Studies of the morphology of tissue specimens as well as cell cultures from normal and abnormal ocular tissues are essential for further insights into possible pathogenetic mechanisms of disease. The utilization of immunohistochemical methods and histochemical stains is also helpful in the diagnosis of certain conditions.

Methods Employed: Specimens are obtained from patients at the National Eye Institute as well as other ophthalmic centers in the United States. In most instances, specimens are processed by appropriate techniques for histology, histochemistry, and electron microscopy. Selected specimens are frozen for special immunological studies. In other cases, routine histopathology is performed.

Major Findings:

I. Histologic studies of selected normal human ocular tissues

Scanning and transmission electron microscopy was performed on normal human cornea, iris and trabecular meshwork obtained from eye bank and autopsy eyes. Cell cultures of the iris and trabecular meshwork were also examined by the same methods.

Three book chapters were prepared for a text book of histology. These included scanning and transmission electron microscopy of the normal human cornea, iris, and developmental abnormalities of the cornea.

II. Langerhans cells in normal conjunctiva and cornea of selected species

The distribution of Langerhans cells (LCs) in human corneal and conjunctival epithelial sheets was investigated by histochemical, immunofluorescence, and microscopic methods. The LCs stained positive for ATPase and with antibodies to HLA-DR antigen and were negative to DOPA-oxidase. Human conjunctiva showed 250 to 300 LCs/mm² compared to 15 to 20/mm² in the peripheral third of the corneal epithelium.

III. Glaucoma

A. Primary open-angle glaucoma

i. Electron microscopy of argon laser therapy in open-angle glaucoma

Scanning and transmission electron microscopy were performed on trabeculectomy specimens from patients with medically uncontrolled progressive primary open-angle glaucoma. Trabeculectomy specimens were obtained three hours to one year after continuous argon laser trabeculoplasty. The laser-treated sites showed irregular areas of disruption or obliterated trabecular beams. In more recent burns, fibrinous material and occasional macrophages were present. Specimens examined after a longer interval following laser showed considerable fibrosis at the treated sites. The laser burns primarily involved superficial

and midtrabecular meshwork did not extend to Schlemm's canal.

ii. Electron microscopy of acute elevation in intraocular pressures following argon laser therapy in open-angle glaucoma.

Trabeculectomy and iridectomy specimens excised from five patients with persistent medically unresponsive elevation of pressure following argon laser trabeculoplasty and argon laser iridotomy were studied by light and electron microscopy. Histological examination disclosed macrophages, lymphocytes, activated trabecular endothelial cells, and tissue debris from trabecular beams. This inflammatory response and tissue debris initiated by laser treatment appears to be an important factor in the decompensation of the aqueous outflow system.

B. Chandler's syndrome

Cases of Chandler's syndrome were characterized clinically by unilateral glaucoma, mild iris stromal atrophy, corneal endothelial dystrophy, and elevated intraocular pressure. They were examined by slit lamp microscopy and gonioscopy and had photographic documentation of the significant changes. Scanning and transmission electron microscopy of corneas, trabeculectomy and iridectomy specimens disclosed a downgrowth of degenerated corneal endothelium and Descemet's membrane across the inner uveal meshwork. The iris stromal changes were minimal and the corneal endothelial extension across the trabecular meshwork disclosed a moderate increase of microvilli, cytoplasmic blebs, and filopodial processes. Descemet's membrane was irregularly thinned and closely adherent to the inner uveal meshwork.

Immunohistologic stains were performed with antibodies to human epidermal keratin to further distinguish cases of Chandler's syndrome from posterior polymorphous dystrophy.

IV. Vitreoretinal disorders

A. Epiretinal membranes

Periretinal membranes obtained at vitrectomy from three patients with massive periretinal proliferation were examined by immunofluorescence and electron microscopy. Immunofluorescent staining on fresh frozen sections showed positive stain with glial fibrillary acidic protein and a weak stain with antibodies to actin, PBM-1 and laminin. Staining was negative for antibodies to collagen types I, II and IV. Electron microscopy revealed abundant glial cells arranged in a tubulo-acinar configuration with junctional complexes, apical microvillous processes and 9-10 nm cytoplasmic filaments.

B. Retinitis pigmentosa: Electron microscopy and cell culture

We observed unusual changes in the retinal pigmented epithelium (RPE), predominantly of the macula and foveal region, in addition to the more commonly observed degeneration of photoreceptor cells. Scanning electron microscopy of cell cultures of the RPE cells showed a variety of cell types, some compatible with those seen in cultures of normal human RPE (Flood, Gouras, and Kjeldbye,

as well as other distinctly abnormal cells.

V. Ocular manifestations of systemic diseases

i. A thirty-five year old homosexual male with cytomegalovirus viremia developed retinitis. He also had a new syndrome consisting of a persistent T lymphocyte deficit, pneumocystitis pneumonia, recurrent Candida esophagitis, skin ulcerations caused by Herpes simplex virus, type 2 disseminated Mycobacterium avium-intracellulare infection, and molluscum.

ii. Leishmaniasis affecting the eyelids

Leishman bodies were recognized in the smear of a biopsy from an eyelid ulcer. The infecting organisms were identified serologically as Leishmania braziliensis panamensis. The ulcer responded to pentavalent antimony. Ultra-structurally, the organisms had double-unit membranes beneath which lay a palisade of microtubules. At one end of the organism, there was a rudimentary flagellum; at the other, the nucleus. A kinetoplast basal complex separated the two.

iii. Gaucher's disease

Several reports state (1) that yellowish-brown pingueculae are the characteristic ophthalmic sign of Gaucher's disease (GD) and (2) that the lesions are infiltrated with Gaucher's cells.

We studied 73 patients with GD. Forty-four had no evidence of pingueculae; in 27, they were obvious; and 2 had unilateral conjunctival discolorations suggestive of the lesion. The incidence of pingueculae in GD are no different than those in the general population.

VI. Ocular and adnexal tumors

A thirty-seven year old white female with xeroderma pigmentosum had reduced vision for many years because of primary and secondary corneal epithelial edema and stromal haze. Corneal grafting was required, but was not successful. Numerous primary dermal tumors of various types involving the lids of both eyes had been surgically excised or treated by freezing with liquid nitrogen. Squamous cell carcinoma involving the limbal area of the globe and adjacent tissues were excised from the left eye at age twelve, the right eye at age thirty-two and the left eye (again) at age thirty-six. The right limbal tumor soon recurred and invaded the orbit despite radiation treatment; this required right orbital exenteration. The second left limbal tumor recurred one year later, soon after the recurrence of a left lower lid basal cell carcinoma. Left orbital extenteration was required. Corneal graft failure and recurrent ocular squamous cell carcinoma involving the eye in the xeroderma pigmentosum can be difficult management problems.

Significance to Biomedical Research and the Program of the Institute:
These studies are directly concerned with mechanisms involved in primary and secondary glaucoma, corneal, conjunctival and retinal as well as ocular manifestations of systemic diseases.

Proposed Course: These projects will continue in the next fiscal year.

NEI Research Program: Glaucoma--Primary Open-Angle Glaucoma; Corneal Diseases--External Ocular infections and inflammatory Diseases; Retinal and Choroidal Diseases--Diabetic Retinopathy, Sickle Retinopathy and other Vascular Abnormalities.

Publications:

Rodrigues MM, Rowden G, Hackett J, Bakos I: Langerhans cells in the normal conjunctiva and peripheral cornea of selected species. Invest Ophthalmol Vis Sci 21:759-765, 1981.

Rodrigues MM, Newsome DA, Machemer R: Further characterization of epiretinal membranes in human massive periretinal proliferation. Curr Eye Res 1:311-315, 1981.

Bonney CH, Gaasterland DE, Rodrigues MM, et al: Acute effect of Q-switched ruby laser on monkey anterior chamber angle. Invest Ophthalmol Vis Sci 22:310, 1982.

Chu F, Rodrigues MM, Cogan DG, Neva FA: Leishmaniasis affecting the eyelids. Arch Ophthalmol (in press).

Bachman DA, Rodrigues MM, Chu FC et al: Culture-proven cytomegalovirus retinitis in a homosexual male with acquired immunodeficiency syndrome. Ophthalmology (in press).

Gaasterland D, Rodrigues M, Moshell A: Ocular changes in xeroderma pigmentosum. Ophthalmology (in press).

Rodrigues MM, Newsome DA: Retinitis pigmentosa: Electron microscopy and cell culture studies. Proceedings of the International Symposium on Genetics in Ophthalmology, New York. Alan R. Liss, Inc. (in press).

PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Histopathologic Studies of Animal Models of Human Ocular Diseases

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Merlyn M. Rodrigues	M.D.	Chief, Section on Clinical Eye Pathology	CB	NEI
Other:	Sanford Meyers	M.D.	Senior Staff Ophthalmologist	CB	NEI
	Carol Currier	M.D.	Senior Staff Fellow	CB	NEI

COOPERATING UNITS (if any)

LAB/BRANCH
Clinical BranchSECTION
Section on Clinical Eye Pathology

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0.2 PROFESSIONAL: 0.1 OTHER: 0.1

CHECK APPROPRIATE BOX(ES)

 (a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER (a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A model of virus-induced diabetes mellitus showed oculo-renal changes that were similar to human diabetes. Fundus lesions in monkeys, both acute and long term, were evaluated after carotid injection of bacteria. A rabbit model was used to test the effect of selected intravitreal drugs after severe penetrating injury.

Project Description:

Objectives: Light and electron microscopic examinations of eye and other tissues in these models provide essential information related to the course of the disease and type of cellular inflammation responses.

Methods Employed:

1. Virus-induced diabetes in mice: (DBA, NZB and SJL strains). Mice infected with the D (diabetogenic variant of the M strain of the encephalomyocarditis EMC) virus resulted in diabetes monitored by hyperglycemia and glycosuria within one month. Light microscopic examination was performed on the eyes and kidneys.

2. Five pigtail monkeys (Macaque nemestrina) had seven carotid injections of S. mutans suspensions, 10 cc of 5×10^7 organisms/cc in four monkeys and 10 cc of $\sim 5 \times 10^8$ organisms/cc in three monkeys. Two of the monkeys had bilateral carotid injections several weeks apart.

3. Effect of selected drugs after severe penetrating injury in rabbits
Double-penetrating posterior wounds with vitreous hemorrhages were produced with a 13-gauge needle in rabbit eyes. Following bilateral injury to five rabbits in a randomized assignment, one eye had 0.0. mg (0.1 cc) intravitreal injection of cytosine arabinoside, Adriamycin, dexamethasone or doxorubicin in sterile normal saline; the other eye had a 0.1 cc intravitreal injection of sterile normal saline.

Major Findings:

1. Oculo-renal changes in EMC virus-induced diabetes mellitus in mice: Diabetic mice with the longest duration (six months) of diabetes showed the most marked alterations. Fasting blood sugar levels were 320-395 mg/dl and glycosuria was present. Clinically, based on ophthalmoscopy and fluorangiograms retinal vessels were normal; the only abnormality was decreased numbers of pericytes by trypsin digestion. Corneal epithelial edema was present and surface microvillus projections were decreased compared to controls. The kidneys of the same diabetic animals showed nodular and diffuse glomerulosclerosis and mesangial thickening similar to human Kimmelstiel Wilson disease. Histologically, moderate to advanced kidney disease was associated with relatively early retinopathy.

2. Fundus lesions after carotid injection of Streptococcus mutans in monkeys: Carotid injection of Streptococcus mutans in pigtail monkeys caused fundus lesions clinically resembling those seen in humans with bacteremia. On histopathological examination microabscesses occurred in the retina, choroid, and optic nerve. Bacteria were observed in the histopathological sections of the microabscesses, and S. mutans was cultured from the retina and choroid.

3. Effect of selected intravitreal drugs after penetrating injury in rabbits: The rabbit model of severe double penetrating posterior injury was used to test the efficacy of several chemotherapeutic agents in preventing vitreal membranes. Intravitreal injection of cytosine arabinoside, doxorubi-

cin, methotrexate, hydroxyurea, or dexamethasone did not alter the frequency or severity of the resultant vitreal membranes. Similarly, intravenous cyclophosphamide, methylprednisolone, or doxorubicin were not effective.

Significance to Biomedical Research and the Program of the Institute: In virus-induced diabetes mellitus the renal and ocular changes appear similar to those seen in man and will be investigated further. The S. mutans model provides an opportunity to study acute and chronic changes in the fundus that simulate human disease.

Proposed Course: These projects will continue in the next fiscal year.

NEI Research Program: Retinal and Choroidal Diseases--Tissue Acquisition and Distribution; Human Donor Eyes and Animal Models/Diabetic Retinopathy; Inflammatory Disorders.

Publications:

Yoon J, Rodrigues MM, Currier CA, Notkins AL: Virus-induced diabetes mellitus in mice: long term complications. Nature 296:566, 1982.

Meyers SM, Rodrigues MM, Vasil ML: Fundus lesions after carotid injection of Streptococcus mutans in monkeys. Infect Mutans 35:1079, 1982.

Meyers SM, Rodrigues MM: Effect of selected intravitreal drugs after severe penetrating injury in rabbits. Curr Eye Res 1:471, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00030-11 CB

PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Studies of Parameters of Intraocular Pressure

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Douglas E. Gaasterland	M.D.	Chief, Section on Glaucoma	CB	NEI
Other:	Carl Kupfer	M.D.	Director		NEI
	Lessie McCain	R.N.	Clinical Technician	CB	NEI
	Roy Milton	Ph.D.	Chief, Section on Biometry	OBE	NEI
	Elmer J. Ballantine	M.D.	Clinical Director	CB	NEI

COOPERATING UNITS (if any)

Pharmaceutical Development Service, CC, NIH
Department of Ophthalmology, Mayo Clinic, Rochester, Minnesota

LAB/BRANCH

Clinical Branch

SECTION

Section on Glaucoma

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

0.10

PROFESSIONAL:

0.05

OTHER:

0.05

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS

(b) HUMAN TISSUES

(c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Young and old normal volunteers and patients with glaucoma or ocular hypertension participate in this continuing study of the parameters of intraocular pressure. The acute and long-term effects of antiglaucoma medications alone and in combination upon the parameters are studied in normal and in diseased eyes.

Project Description:

Protocol Number: 75 EI 114

Objectives: To evaluate parameters of intraocular pressure and aqueous dynamics in normal eyes and eyes with ocular hypertension or glaucoma before and after medications.

Methods Employed: Replicate studies are done upon experienced human participants. Seven parameters are determined before and after medication: intraocular pressure, episcleral venous pressure, total facility, true facility of outflow, pseudofacility, aqueous flow, and ocular rigidity. Acute drug effects are emphasized. Aqueous flow is measured noninvasively by fluorimetry.

Major Findings: During FY 82 the principle activity in this project has been initiation of a study of the effect of isoproterenol on aqueous flow as measured fluorimetrically. This has been done in collaboration with Richard F. Brubaker, M.D. at the Mayo Clinic. A protocol for the study was prepared and approved by the Mayo Clinic Human Research Review Panel. Permission was obtained to extend our investigational new drug coverage for topical isoproterenol to include this study. Normal volunteer participants receive either topical fluorescein by iontophoresis or systemic fluorescein in this acute study. One eye receives 2 percent d,l-isoproterenol hydrochloride and the other eye receives vehicle. Medications have been prepared in a masked manner and neither the technician nor the patient knows which eye receives medication. Analysis of measurements from seven patients has shown a small decrease of intraocular pressure, but there was no effect upon fluorimetrically measured aqueous humor flow or anterior chamber turnover constant. Therefore, the studies with 2 percent isoproterenol were discontinued and 4 percent d,l-isoproterenol hydrochloride prepared. FDA approval to extend our investigational new drug clearance to 4 percent concentration was obtained. Studies with masked 4 percent medication vehicle combinations have started.

Significance to Biomedical Research and the Program of the Institute: Study of changes of the parameters of intraocular pressure caused by glaucoma medications allows clearer understanding of the mechanisms of action. Studies of these parameters more clearly define the difference between normal and abnormal. The measurements can be extrapolated to more basic physiologic functions, yielding insight to the function of the human eye.

Proposed Course: The project will be continued, emphasizing medication effects upon flow of aqueous humor.

NEI Research Program: Glaucoma--Primary Open-Angle Glaucoma--Aqueous Humor Dynamics: Inflow/Aqueous Humor Dynamics: Outflow

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00143-09 CB

PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Radioiodinated Chloroquine Analog for Diagnosis of Ocular Melanoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Douglas E. Gaasterland	M.D.	Chief, Section on Glaucoma	CB	NEI
Other:	Elmer J. Ballantine	M.D.	Clinical Director	CB	NEI
	Carl Kupfer	M.D.	Director		NEI

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Branch

SECTION

Section on Glaucoma

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

0.02

PROFESSIONAL:

0.02

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS

(b) HUMAN TISSUES

(c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Thirty-six patients with pigmented ocular lesions originally participated in this study. The early results of the study show that the diagnostic technique had inadequate specificity. For most patients a clear clinical diagnosis has been made, and their ocular problem resolved. Except for occasional follow-up examinations of some of the patients, work on this project has ended.

Project Description:

Protocol Number: 76 EI 370

Objectives: To determine the value of using I-125 labeled chloroquine analog for the detection of ocular melanoma.

Methods Employed: Follow-up clinical examinations are performed.

Major Findings: One of the patients with a lesion originally thought to be benign has had another examination after eight years in the study. There was no change in the diagnosis. Her large, flat, pigmented, foveal nevus has now been under followup examination for more than 25 years. A patient with a pigmented mass lesion originally regarded as suspicious continues to have no increase in size of the lesion after eight years of followup. A patient with an iris melanoma has a dense cataract but no change of the tumor. The tumor involves the anterior chamber angle, but has not changed in more than 12 years. Two patients who have repeatedly refused enucleation continue to show slow growth of choroidal melanomas. There is no evidence of metastatic disease eight and nine years after diagnosis. In one, visual function remains good. He is now in his fifties. The other patient recently lost vision in the eye, and subsequently developed iris neovascularization with hemorrhagic glaucoma. She is now 87 years old, and continues to refuse enucleation.

Significance to Biomedical Research and the Program of the Institute:
Continued follow-up of the patients is important because it gives information about the course of the disease with and without treatment.

Proposed Course: The intermittent examination of this small group of patients will be continued.

NEI Research Program: Retinal and Choroidal Diseases--Tumors

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZOE EY 00154-09 CB

PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Experimental Glaucoma in the Rhesus Monkey

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Douglas E. Gaasterland M.D. Chief, Section on Glaucoma CB NEI
Other: Merlyn Rodrigues M.D. Chief, Section on Clinical CB NEI
Eye Pathology

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Branch

SECTION

Section on Glaucoma

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.02	0.02	0.0

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this investigation is to study the morphology, physiologic function, and pharmacologic responses in the eye of the rhesus monkey in its normal state compared to its state when experimental glaucoma has been induced by argon laser photocoagulation of the trahecular meshwork.

Project Description:

Objectives: To study physiologic function, pharmacologic responses, and morphology of the monkey eye after induction of glaucoma by argon laser photo-coagulation of the trabecular meshwork. To compare observations to normal control eyes.

Methods Employed: Circumferential argon laser photocoagulation of the rhesus monkey trabecular meshwork causes sustained elevation of intraocular pressure to the range of 30 to 55 mmHg, the pressure range found in many humans with open-angle glaucoma. This is in contrast to the acute, short duration, very high pressure elevation (more than 65 mmHg, up to 95 mmHg) seen in most animal models for glaucoma. Outflow facility has been evaluated by perfusion. Aqueous flow has been determined by turnover of radioiodinated serum albumin injected into the anterior chamber. Retinal and optic nerve function have been studied clinically and by autoradiography and morphologically to evaluate evidence of altered axoplasmic flow. The retina has been studied in cross section and by preparing whole-mounts of the tissue. Studies of the effect of less than circumferential argon laser photocoagulation have been done.

Major Findings: In FY 1982 the study of the effect of treatment of less than the full extent of the circumference of the anterior chamber angle was discontinued. Tissue was examined from two monkeys. These monkeys had numerous, confluent 50 micron burns which were applied using 0.6 watts and 0.2 to 0.5 seconds duration. Three of the four eyes of the two monkeys were treated. In one eye, 50 percent of the circumference was treated on two occasions and in the other eye of the same monkeys three-quarters of the circumference was treated on two occasions. The interval between the two treatments was six months. In the third treated eye, 90 percent of the circumference was treated once. Untreated areas and the fourth eye of the second monkey served as controls. Followup for the two eyes with repeat treatment was three years after the second treatment, and for the eye with the single treatment was nine months. Light microscopy, transmission and scanning electron microscopy show conventional outflow pathway scarring. There is distortion of the treated area with reactive cellular changes. Endothelial cells with prominent filopodial processes extend a variable distance from the inner corneal surface across scarred trabecular meshwork. The reactive changes are particularly noticeable in the three year specimens. Untreated areas are normal.

Significance to Biomedical Research and the Program of the Institute: This experimental glaucoma is a model for human chronic open-angle ("simple") glaucoma. Using this model allows examination of retina and optic nerve changes caused by glaucoma, and may give insight into the mechanism of loss of visual function in the patient with glaucoma. This work has also yielded information about the effect of argon laser on the simian outflow pathway.

Proposed Course: The project is being completed.

NEI Research Program: Glaucoma--Primary Open-Angle Glaucoma/Secondary Glaucomas

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00168-07 CB
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PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Laser Surgery for Glaucoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Douglas E. Gaasterland M.D.	Chief, Section on Glaucoma	CB	NEI
Other:	Charles Bonney D.V.M, Ph.D.	Visiting Scientist	CB	NEI
	Elmer J. Ballantine M.D.	Clinical Director	CB	NEI
	Robert Bonner Ph.D.	Physicist	BEIB	DRS
	Claude Cummins B.S.	Biologist	CB	NEI
	Alan H. Rich B.S.	Engineer	BEIB	DRS
	Sumana K. Davi Ph.D.	Expert	CB	NEI
	Gerald W. Liesegang Ph.D.	Senior Staff Fellow	IR-TD	NHLBI
	Merlyn Rodrigues M.D., Ph.D.	Chief, Section on Clinical Eye Pathology	CB	NEI

COOPERATING UNITS (if any)

Biomedical Engineering and Instrumentation Branch, DRS; Armed Forces Radiobiology Research Institute

LAB/BRANCH

Clinical Branch

SECTION

Section on Glaucoma

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.31	1.21	0.1

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The high energy and power of lasers offer a tool for noninvasive alteration of anterior intraocular tissue. Specifically, iridotomy and trabeculotomy are possible. This has importance for glaucoma patients because of the potential improvement of surgical outcome and reduced surgical morbidity. The aim of this project is a systematic evaluation of laser effects in simian (rhesus) eyes and the application of promising systems and procedures to human glaucoma eyes under controlled conditions.

Project Description:

Protocol Number: 80 EI 91, 81-EI-204

Objectives: To develop workable laser systems for anterior segment surgery and to apply these systems to the normal monkey eye. To study the physiological and morphologic effects of laser energy upon monkey eyes. To apply favorable laser systems under controlled conditions to the treatment of glaucoma in humans.

Methods Employed: Instruments are being developed to meet the unique requirements of ophthalmic application. Standard laboratory physiologic and histopathologic (including light microscopy, SEM and TEM) techniques are employed to study laser effects. The NEI laser laboratory is equipped with a modified model 800 Coherent Radiation argon laser, which has been used for this and other projects, a Q-switched ruby laser, two Q-switched neodymium-YAG lasers, and delivery systems.

Major Findings: During FY 82, Sumana K. Davi, Ph.D., joined this project as an expert to provide skills required for development and testing of instrumentation. Instrument development has continued to be an important activity during FY 82. The Advanced Kinetics light guide for delivery of the Q-switched ruby laser pulses to the slitlamp delivery system proved too heavy and difficult to keep in alignment for a clinical delivery system. It has been modified, but must be replaced. The Coherent Radiation Company, Medical Division, donated two articulated arms from Model 800 argon laser photocoagulators to the laboratory. These are being modified, by replacement of the mirrors, to deliver pulsed laser energy. Alignment of these arms after insertion of the new mirrors has proven to be beyond our on-campus capabilities. We have arranged that this be done at the Coherent Company on their optical bench. A 2X magnification beam expander has been installed into the ruby laser delivery system by fabrication of a holder that attaches to the slitlamp. This allows an improvement in the F-number of the system, a smaller spot size, and a larger beam cone-angle.

Initial design and fabrication of the parts required for delivery of pulses from the neodymium-yag system have been finished. It is anticipated this system will be put into operation in FY 83.

Discussions with staff of the Coherent Radiation Company led to sharing of our ideas concerning design of a laser treatment system using a small, range finder-type, laser. These neodymium-yag lasers put out up to 25 millijoules per pulse in 10 to 15 nanoseconds. If focused, this would lead to sufficient power to create local, non-linear effects. Coherent has designed and built a prototype instrument. This prototype has been delivered to our laboratory for testing and comparison to our Q-switched ruby laser system.

A protocol (No. 81-EI-204) was prepared for an investigation comparing Q-switched ruby laser pulse discussion to surgical discussion in patients with pupillary membranes. Eligible patients are randomly assigned to one of the two treatment modalities. Two patients have entered the study. Both were assigned to laser treatment. One treatment was done and there is now a six month

followup. Opening the membrane in the pupil was successful. Vision improved immediately from 20/200 to 20/25. The second patient will be treated next week. An additional five patients who were referred, examined, and found not eligible for the study elected to have laser dissection of pupillary membranes outside the study. In all cases this was successful. Complications include transient elevation of intraocular pressure in two of the five and discrete peripheral retinal burns with pinpoint hemorrhages in three of the five. These burns are located along the line of aiming. The burns start to become hyperpigmented in one week. The burns of the retina are thought to be explained by the seven degree cone-angle of the Q-switched ruby laser system. It is believed this problem will be alleviated by the newer systems which have a three times larger cone angle. Improved instrumentation will also allow easier cutting of the membranes.

Intraocular lens damage threshold is being assessed in a related, in vitro study. Intraocular lenses are damaged by 200 micron diameter pulses of 20 to 30 nanosecond duration containing three to ten millijoules per pulse from the Q-switched ruby laser. With a mode-locked, frequency-doubled neodymium glass system, spot sizes of 400 micron and pulse durations of 30 picoseconds result in injury to the lens when the pulse energy exceeds three millijoules. The morphology of these lens lesions differs. These studies will be expanded to better define the mode-locked, frequency-doubled and primary frequency damage threshold for intraocular lenses and to examine the effect upon the damaged threshold of immersion of the lenses. All studies to date have been done with the lenses in air.

The clinical study comparing argon laser trabecular treatment to surgical trabeculectomy (protocol 80-EI-91) has been continued. Recruitment has proven difficult as patients and physicians in the community have come to prefer argon laser treatment to surgery for the initial intervention in patients requiring treatment beyond medications. Nine patients have now entered the study and followup ranges from six months to greater than two years. It has already become clear that the surgical effect upon intraocular pressure exceeds that of laser trabecular treatment, but that this is obtained at the cost of a higher incidence of cataracts and more complex early post-treatment healing problems. Patients require fewer medications after trabeculectomy than after laser treatment to control intraocular pressure within a clinically acceptable range.

Significance to Biomedical Research and the Program of the Institute:
Conceivably, a physically-noninvasive laser system for anterior segment surgery could replace conventional invasive operative procedures for treating some types of glaucoma and other anterior segment problems. This possibility is being investigated.

Proposed Course: The project will continue. Instrument development will include adaptation of pulsed lasers to the operating microscope and the slitlamp using various delivery configurations. The clinical trials will be continued and expanded.

NEI Research Program: Glaucoma--Primary Open-Angle Glaucoma/Angle-Closure Glaucoma/Developmental, Congenital, or Infantile Glaucoma/Secondary Glaucomas/ Aqueous Humor Dynamics: Outflow

Publications:

Bonney CH, Gaasterland DE, Rodrigues MM, Raymond JJ, Donohoo P: Short-term effects of Q-switched ruby laser on monkey anterior chamber angle. Invest Ophthalmol Vis Sci 22:310-318, 1982.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER		
PERIOD COVERED October 1, 1981, to September 30, 1982					
TITLE OF PROJECT (80 characters or less) Laboratory Studies of Aqueous Humor Dynamics					
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT					
PI:	Douglas E. Gaasterland	M.D.	Chief, Section on Glaucoma	CB	NEI
Other:	John A. Barranger	M.D.	Chief, Clinical Section	DMNB	NINCDS
	Toichiro Kuwabara	M.D.	Chief, Section on Experimental Pathology	LVR	NEI
	Pamela Robey	Ph.D.	Staff Fellow	CB	NEI
	Claude C. Cummins, III	BS	Summer Student (Biologist)	CB	NEI
COOPERATING UNITS (if any) Developmental and Metabolic Neurology Branch, NINCDS, NIH					
LAB/BRANCH Clinical Branch					
SECTION Section on Glaucoma					
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205					
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:			
0.05	0.05	0.0			
CHECK APPROPRIATE BOX(ES)					
<input type="checkbox"/> (a) HUMAN SUBJECTS		<input type="checkbox"/> (b) HUMAN TISSUES		<input checked="" type="checkbox"/> (c) NEITHER	
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS					
SUMMARY OF WORK (200 words or less - underline keywords)					
<p>Investigations have been done to clarify <u>intraocular fluid movement</u> in <u>rhesus monkeys</u> and <u>humans</u>. A method was perfected for spectrophotometric determinations of <u>ascorbic acid concentration</u> in ocular and systemic fluids. This is being applied to human aqueous samples. Monkey <u>aqueous humor</u> has been analyzed for <u>glycosaminoglycan</u>, <u>glycoprotein</u> and <u>hyaluronidase</u> content.</p>					

Project Description:

Objectives: The goal of this project is to clarify aspects of intra-ocular fluid movement.

Methods Employed: Standard methods of eye and vascular cannulation, of perfusion, and of noninvasive and invasive pressure measurements, with determination of volumes and flow by weight changes, dilution or turnover techniques have been used. Aqueous obtained by ocular cannulation is analyzed for concentrations of constituents.

Major Findings: During FY 1982, there have been three activities on this project. 1) Data previously obtained has been prepared for publication. 2) A pooled sample of normal rhesus monkey aqueous humor was obtained from sixteen eyes of eight animals. The sample contains combined anterior and posterior aqueous humor. The pooled sample was divided and a portion submitted for analysis of content of glycosaminoglycans and hyaluronic acid. None was found. The remaining larger aliquot of the sample was concentrated and the analysis performed again. Still no glycosaminoglycans or hyaluronic acid were found. Another pooled sample of aqueous humor from additional animals is being analyzed for hyaluronidase content. The results are not yet available. 3) A study of the aqueous drainage pathways has been started. Batson's #17 plastic embedding compound is microinjected into the episcleral veins. This flows retrograde to the collector channels and canal of Schlemm. Early efforts in this project have involved learning proper methodology with a plastic compound to achieve blood-like viscosity for use during injection.

Significance to Biomedical Research and the Program of the Institute: The studies are elucidating normal dynamics of aqueous humor, as well as abnormal dynamics in experimentally induced situations, mimicking clinical problems. These studies are yielding information applicable to understanding and treating glaucoma and hypotony.

Proposed Course: Biochemical and anatomic studies will continue at a low level of activity.

NEI Research Program: Glaucoma--Primary Open Angle Glaucoma--Aqueous Humor Dynamics: Inflow/Aqueous Humor Dynamics: Outflow.

Publications:

Gaasterland DE, Barranger JA, Rapoport SI, Girton ME, Doppman JL: Longer-term ocular effects of osmotic modification of the blood-brain barrier in monkeys. I. clinical examinations; aqueous ascorbate and protein. Invest Ophthalmol Vis Sci (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00050-06 CB
PERIOD COVERED October 1, 1981, to September 30, 1982			
TITLE OF PROJECT (80 characters or less) Aqueous Humor Flow Measurement by Fluorophotometry			
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Douglas E. Gaasterland M.D. Chief, Section on Glaucoma CB NEI Other: Lessie McCain R.N. Clinical Technician CB NEI			
COOPERATING UNITS (if any)			
LAB/BRANCH Clinical Branch			
SECTION Section on Glaucoma			
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205			
TOTAL MANYEARS: 0.01	PROFESSIONAL: 0.01	OTHER: 0.00	
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS			
SUMMARY OF WORK (200 words or less - underline keywords) <u>The aqueous humor flow in humans is measured by determining the rate of loss of fluorescein from the eye after iontophoresis into the cornea in normal volunteers and in patients with ocular hypertension or glaucoma.</u>			

Project No. Z01 EY 00050-06 CB

Project Description:

Protocol Number: 77 EI 104

Objectives: The symmetry and reproducibility of direct measurements of aqueous humor flow in the two eyes of normal volunteers and of patients with either ocular hypertension or glaucoma are being studied; medication effects are assessed.

Methods Employed: A cylindrical piece of polyacrylamide gel is saturated with fluorescein solution. The gel is touched to the cornea, and fluorescein is deposited due to a small current provided by a dry cell battery. A photo-multiplier tube with appropriate filters, mounted on a slitlamp biomicroscope, measures the total amount of fluorescein in the eye as well as the aqueous concentration. Illumination is provided by a chopped light source. The photo-multiplier tube signal is fed to a tuned amplifier. The rate of loss of fluorescein from the eyes as a function of time yields the flow rate of aqueous humor.

Major Findings: The major activity on this project during FY 82 was review of data previously obtained to assess symmetry and reproducibility. This information and the effects of ocular hypertension upon flow are being prepared for publication. Mean flow of aqueous is symmetric in groups of normal volunteers and of ocular hypertension patients. The effects of isoproterenol upon aqueous flow have been studied in project Z01 EY 00030-10 CB "Studies of Parameters of Intraocular Pressure" with Richard F. Brubaker, M.D. at the Mayo Clinic, Rochester, Minnesota.

Significance to Biomedical Research and the Program of the Institute: The aqueous humor flow rate is a primary determinant of the intraocular pressure. This accurate, safe, reproducible, noninvasive, direct determination of the flow in humans under normal and pathological conditions has helped in the understanding of glaucoma and hypotony.

Proposed Course: This project is being terminated.

NEI Research Program: Glaucoma--Primary Open-Angle Glaucoma--Aqueous Humor Dynamics: Inflow/Aqueous Humor Dynamics: Outflow

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00077-05 CB
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PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Treatment of Neovascular Glaucoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Douglas E. Gaasterland M.D.	Chief, Section on Glaucoma	CB NEI
Other: Elmer J. Ballantine M.D.	Clinical Director	CB NEI

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Branch

SECTION

Section on Glaucoma

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

0.00

PROFESSIONAL:

0.00

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

 (a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER (a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Patients with rubeosis iridis and neovascular glaucoma are being recruited. Those with salvageable vision are invited to join this prospective, randomized study of whether cyclocryotherapy or cyclodiathermy is better for the treatment of this disease. Outcome will be judged by assessing preservation of visual function; adequate control of intraocular pressure, with or without medications; and control of discomfort. It is estimated that approximately 40 nondiabetic and 40 diabetic patients are needed for this project.

Project No. Z01 EY 00077-05 CB

Project Description:

Protocol Number: 78 EI 17

Objectives: To determine whether one of two methods for ciliary body ablation, cyclodiathermy or cyclocryotherapy, is better for treatment of neovascular glaucoma.

Methods Employed: Patients who are eligible to join the study, and who consent to participate, are randomly assigned to receive one of the two methods of treatment. Follow-up is aimed at identifying adequacy of treatment and identifying complications.

Major Findings: No new patients have entered the study during FY 1982.

Significance to Biomedical Research and the Program of the Institute: This study has potential for indicating the proper management of these difficult secondary glaucoma patients.

Proposed Course: The study will be continued to allow gathering of additional data.

NEI Research Program: Glaucoma--Secondary Glaucomas

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00086-04 CB
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PERIOD COVERED
October 2, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Contributions to Ophthalmic Pathology and Systemic Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: David G. Cogan	M.D.	Chief, Neuro-Ophthalmology Section	CB	NEI
Other: Toichiro Kuwabara	M.D.	Chief, Experimental Pathology Section	LVR	NEI
Merlyn Rodrigues	M.D.	Chief, Ophthalmic Section	CB	NEI
Fred C. Chu	M.D.	Senior Staff Fellow	CB	NEI
David M. Bachman	M.D.	Senior Staff Fellow	CB	NEI
W. Gerald Robison	M.D.	Geneticist, Cell Biologist	LVR	NEI

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Branch

SECTION

Neuro-Ophthalmology Section

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0.4	PROFESSIONAL: 0.3	OTHER: 0.1
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CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Tissue obtained either by biopsy or necropsy is studied with the aim of elucidating clinical signs and symptoms. Specific studies included: (1) the pathology and virology of the acquired immunodeficiency (AID) syndrome, and (2) corneal changes with abnormal lipoproteinemia.

Project No. Z01 EY 00086-04 CB

Project Description:

Objectives: To interpret ophthalmic manifestations of disease through the study of pathologic processes in tissues and associated clinical signs during life.

Methods Employed: Tissue obtained by biopsy or necropsy are subjected to microscopy and, where indicated, to electron microscopy.

Major Findings: (1) A clinico-pathologic correlation of the newly identified AID syndrome (acquired immunodeficiency) in which the cytomegalic virus was shown to be the cause of the blinding retinopathy.
(2) Documentation of lipid deposition in the corneas of patients with abnormal lipoproteinemias. (In conjunction with Ernst Schaefer, M.D.)

Significance to Biomedical Research and the Program of the Institute: Tissue changes provide the traditional means of understanding disease. Clinicians must have an awareness of, and access to, pathologic material in order to interpret clinical signs.

Proposed Course: To take full advantage of opportunities to study patients and tissue that become available and to report the results to colleagues who are involved in patient care.

NEI Research Program: Retinal and Choroidal Diseases--Inflammatory Disorders.

Publications:

Cogan DG: Stroke, in Van Dalen JTW, Lessell S (eds): Neuro-Ophthalmology II. Amsterdam, Excerpta Medica, 1982, pp 280-287.

Cogan DG: The Ocular fundus and hypertension, in Amery (ed): Hypertensive Cardiovascular Disease: Pathophysiology and Treatment. The Hague, Martinus Nijhoff, 1982, pp 405-419.

Bachman DM, Rodrigues MM, Chu FC, Straus SE, Cogan DG, Macher AM: Culture-proven cytomegalovirus retinitis in a homosexual male with the acquired immunodeficiency syndrome. Ophthalmology 89:797, 1982.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00087-04 CB
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PERIOD COVERED October 1, 1981, to September 30, 1982
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TITLE OF PROJECT (80 characters or less)
--

Parametric Studies of the Pupillary Functions

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: David G. Cogan	M.D. Chief, Neuro-Ophthalmology Section	CB NEI
Other: Fred C. Chu	M.D. Senior Staff Fellow	CB NEI
Douglas B. Reingold	M.A. Computer Specialist	CB NEI
J. Christian Gillin	M.D. Chief, Unit on Sleep Studies	BPH NIMH
Richard Lowenstein	M.D. Staff Psychiatrist	BPH NIMH
Natraj Sitaram	M.D. Staff Psychiatrist	BPH NIMH
John Nurnberger	M.D. Senior Staff Fellow	BPH NIMH
Elliot Gershon	M.D. Chief of Psychogenetics Section	BPH NIMH

COOPERATING UNITS (if any)

Section on Psychogenetics, Biological Psychiatry Branch, National Institute of Mental Health

LAB/BRANCH

Clinical Branch

SECTION

Neuro-ophthalmology Section

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
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1.1

0.7

0.4

CHECK APPROPRIATE BOX(ES)

<input checked="" type="checkbox"/> (a) HUMAN SUBJECTS	<input type="checkbox"/> (b) HUMAN TISSUES	<input type="checkbox"/> (c) NEITHER
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<input checked="" type="checkbox"/> (a1) MINORS	<input type="checkbox"/> (a2) INTERVIEWS
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SUMMARY OF WORK (200 words or less - underline keywords)
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Pupillary dysfunction is an important criterion for evaluating neuro-ophthalmological disorders. Incidental observations have been made on individual patients but no integrated study has been attempted this past year.

Project Description:

Protocol Number: 80-EI-59

Objectives: To measure pupillary reactions in patients with selected diseases and to create a new method for documenting pupillary abnormalities.

Methods Employed: Pupillary reactions in patients and normal subjects are evoked by using visual stimuli and pharmacological agents. Changes in pupillary diameters are recorded on videotape using an infrared camera. Rate and extent of constriction are monitored with dynamic analog readout of pupillary area. Differential rates of constriction and dilation of a pupil along different axes are monitored with a digital reconstruction of dynamic pupil shape.

Major Findings: Routine testing of pupillary functions has been made this past year, but shortage of time and personnel has precluded any systemic study.

Significance to Biomedical Research and the Program of the Institute: There is potential utility for using the pupils as an index of central neurochemical function in various disorders.

Proposed Course: Further studies of the pupil will be done in patients with affective disorders and where indicated as part of a neuro-ophthalmological examination.

NEI Research Program: Strabismus, Amblyopia, and Visual Processing--Ocular Motility and Strabismus--Neuro-Ophthalmology

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 EY 00089-04 CB

PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

The Eye and Metabolic Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David G. Cogan	M.D.	Chief, Neuro-Ophthalmology Section	CB	NEI
Other:	Fred C. Chu,	M.D.	Senior Staff Fellow	CB	NEI
	David M. Bachman	M.D.	Senior Staff Fellow	CB	NEI
	Toichiro Kuwabara	M.D.	Chief, Experimental Pathology Section	LVR	NEI
	W. Gerald Robinson	Ph.D.	Geneticist, Cell Biologist	LVR	NEI
	John Barranger	M.D.	Chief, Clinical Investigation Service	DMN	NINCDS

COOPERATING UNITS (if any)

Development and Metabolic Neurology Branch, NINCDS

LAB/BRANCH

Clinical Branch

SECTION

Neuro-Ophthalmology Section

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.4	0.2

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Characteristic dysfunctions of the visual and eye motor systems occur in certain inborn errors of metabolism. The abnormalities presumably stem from the intracellular accumulation of abnormal storage materials, which are cytotoxic. Clinical and biochemical observations were made on two patients with a rare variant of Niemann-Pick's disease which we have termed the Macula Halo Syndrome.

Project Description:

Objectives: To identify and characterize the ophthalmic abnormalities in metabolic disease with especial emphasis on those affecting the nervous system.

Methods Employed: Appropriate patients referred by ophthalmic and neuro-logic colleagues are screened for visual and ocular motor abnormalities. Those patients who are found suitable for further studies are, subject to their consent, enrolled in a battery of appropriate tests. Abnormalities are regularly documented by photography and/or video taping. Ancillary tests are enzyme assays and conjunctival biopsies when indicated.

Major Findings: Two patients with a crystalloid opacity about the foveola and splenomegaly were found to have a sphingomyeline deficiency. Four other cases were found in the literature. The ocular fundus is so characteristic that we have called it the Macula Halo Syndrome. It appears to be a relatively benign form of the Niemann-Pick category. The basis for the pathology of the retinal opacity has yet to be established.

Significance to Biomedical Research and the Program of the Institute: The accessibility of the eye and the transparency of its media provide a unique opportunity to recognize abnormal deposits resulting from metabolic disease. Ophthalmologists are thus in a privileged position to contribute importantly to the identification of pathologic states, to the monitoring of treatment, and to the elucidation of pathogenetic processes. But the primary essence is to establish the validity of the observed associations and to correlate the ophthalmic abnormalities with those of other disciplines.

Proposed Course: So long as patients with metabolic faults come to our attention and so long as we have the means to make adequate studies we will continue as we have in the past few years.

NEI Research Program: Retinal and Choroidal Disease--Developmental and Hereditary Disorders

Publications:

Cogan DG: The ocular fundus and hypertension, in Amery A (ed): Hypertensive Cardiovascular Disease: Pathophysiology and Treatment. The Hague, Martinus Nijhoff, 1982, pp 405-419.

Cogan DG, Chu FC, Reingold DB, Barranger J: Ocular motor signs in some metabolic disease. Arch Ophthalmol 99:1802-1808, 1981.

Cogan DG, Chu FC, Bachman DM, Barranger J: The DAF Syndrome. Neuro-ophthalmology, 2:7-16, 1981.

Project Description:

Objectives: To interpret ophthalmic manifestations of disease through the study of pathologic processes in tissues and associated clinical signs during life.

Methods Employed: Tissue obtained by biopsy or necropsy are subjected to microscopy and, where indicated, to electron microscopy.

Major Findings: (1) A clinico-pathologic correlation of the newly identified AID syndrome (acquired immunodeficiency) in which the cytomegalic virus was shown to be the cause of the blinding retinopathy.
(2) Documentation of lipid deposition in the corneas of patients with abnormal lipoproteinemias. (In conjunction with Ernst Schaefer, M.D.)

Significance to Biomedical Research and the Program of the Institute: Tissue changes provide the traditional means of understanding disease. Clinicians must have an awareness of, and access to, pathologic material in order to interpret clinical signs.

Proposed Course: To take full advantage of opportunities to study patients and tissue that become available and to report the results to colleagues who are involved in patient care.

NEI Research Program: Retinal and Choroidal Diseases--Inflammatory Disorders

Publications:

Cogan DG: Stroke, in Van Dalen JTW, Lessell S (eds): Neuro-Ophthalmology II. Amsterdam, Excerpta Medica, 1982, pp 280-287.

Cogan DG: The Ocular fundus and hypertension, in Amery (ed): Hypertensive Cardiovascular Disease: Pathophysiology and Treatment. The Hague, Martinus Nijhoff, 1982, pp 405-419.

Bachman DM, Rodrigues MM, Chu FC, Straus SE, Cogan DG, Macher AM: Culture-proven cytomegalovirus retinitis in a homosexual male with the acquired immunodeficiency syndrome. Ophthalmology 89:797, 1982.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 EY 00117-02 CB

PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Oculomotor Disorders in Human Subjects

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David G. Cogan	M.D.	Chief, Neuro-Ophthalmology Section	CB	NEI
Other:	Fred C. Chu	M.D.	Senior Staff Fellow	CB	NEI
	David M. Bachman	M.D.	Senior Staff Fellow	CB	NEI
	Douglas B. Reingold	M.A.	Computer Specialist	CB	NEI
	David S. Zee	M.D.	Neurologist (on sabbatical from Johns Hopkins)	CB	NEI

COOPERATING UNITS (if any)

LAB/BRANCH
Clinical Branch

SECTION
Neuro-Ophthalmology Section

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	3.0	PROFESSIONAL:	1.0	OTHER:	2.0
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CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Unwanted or inadequate eye movements impair vision. By neurological evaluation we can sometimes localize the damage causing eye movement disorders. Our method is to combine in selected cases the best neurological evaluation possible with quantitative recording of eye movement responses to calibrated vestibular, optokinetic, and discrete visual stimuli. Eye movements are recorded by electro-oculography or infrared oculography, and now by electro-magnetic search coils. The results are analyzed by computer. Presently we are involved in studying the adaptability of the ocular motor system to abnormal input, (presumably a cerebellar function), certain blink-saccade dyskinesias and observations on patients with cortically deprived visual systems.

Project Description:

Protocol Number: 77-EI-140

Objectives: Abnormal eye movements occur as prominent features of various neurological diseases affecting the central nervous system. Our objective in this project is to provide evidence on the localizing value of eye movement disorders in neuro-ophthalmic diagnosis.

Methods Employed: In selected patients with metabolic or neurological disorders, we have attempted to quantitate the type and degree of ocular motor abnormality.

In addition to the standard methods of infrared and electro-oculographic recording we are now using the search coil technique by which the movements of a scleral annulus on the eye are recorded in a magnetic field. First used by Robinson for animals and adapted for human beings by Collewyn, this method provides superior indices of eye movement unaffected by blink artifacts or head movement. For our purposes the graphs so attained are correlated with the clinical appearances documented on video.

Major Findings: Whereas our past interest in cerebellar control of eye movements has emphasized pursuit and saccadic systems, our current emphasis is on the adaptability of the eye movements and positions to aberrant visual input. This interest has been sparked by the opportunity to make repeated measurements on a patient with long-standing sixth nerve palsy. By enforced fixation with one or the other eye for periods of several days it has been possible to obtain information on the ocular motor systems adaptability. This in turn is presumed to depend on an intact cerebellar structure.

Another opportunity to study eye movements and other functions in depth was afforded by a young woman with cortical blindness of traumatic origin. Although presenting with the typical clinical manifestation of Riddoch's phenomenon (movement without form perception) the patient did have subtle visual perceptions that are presumed to be of extrastriate origin. These are being documented at the present time.

Observations have also been made on two patients with abnormal blink-saccade synkineses. Normally a saccade of more than approximately 20° is accompanied by a blink of the lids, but the two acts may be voluntarily dis-associated. In the two patients studied, however, a normal saccade could not be made without an associated blink. Previous reports of this phenomenon have attributed it to a sensory inhibition of the saccade but our ocular recordings in the dark have shown it to be a genuine motor phenomenon. Although both patients had lesions in the posterior fossa the neuro-pathologic basis of the disturbance is unclear. A review of patients with the Arnold-

Project No. Z01 EY 00117-02 CB

Chiari malformation has confirmed the significance of downbeat nystagmus but has also indicated the occasional occurrence of such unusual manifestations as periodic alternating nystagmus and spasms of the near reflex.

Significance to Biomedical Research and the Program of the Institute: Quantitation and modelling of ocular motor disturbances aids in diagnosing lesions within the central nervous system and contributes to our knowledge of how the brain programs eye movements.

Proposed Course: The routine measurements of patients with various ocular motor disturbances has been decelerated during the physical translocation of the Laboratory. The new facilities will shortly be established, and it is planned to continue documenting the disorders insofar as patients, time, and resources permit.

NEI Research Program: Strabismus, Amblyopia, and Visual Processing--Ocular Motility and Strabismus--Motor Neuro-Ophthalmic Disorders

Publications:

Chu FC, Reingold DB, Cogan DG: Lid-triggered synkineses. Ophthalmology 88:1019-1023, 1981.

Cogan DG, Chu FC, Reingold DB: Illusory movement of the environment in the presence of normal eye movements, in Honrubia V, et al (eds): Nystagmus and Vertigo: Clinical Approaches to the Patient with Dizziness. New York, Academic Press, 1982, pp 225-230.

Cogan DG, Chu FC, Reingold DB: Ocular signs of cerebellar disease. Arch Ophthalmol 11:755-760, 1982.

Reingold DB: The transitions between saccades and smooth eye movements in Honrubia V et al (eds): Nystagmus and Vertigo: Clinical Approaches to the Patient with Dizziness. New York, Academic Press, 1982, pp 287-295.

Cogan DG: Ophthalmoplegia, retinitis pigmentosa, deafness, mental retardation and cerebellar symptoms (Kearn-Sayre), in Myrianthopoulos NC (ed): Handbook of Clinical Neurology, Volume 43, "Neurogenetic Disorders, 1981, Part 1.

Cogan DG: Internuclear ophthalmoplegia, in Neetens A (ed): Disorders of Myelin, Belgium (in press).



SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00011-08 CB

PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Pigment Dispersion With and Without Glaucoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Muriel I. Kaiser-Kupfer M.D.	Chief, Section on Ophthalmic Genetics and Pediatric Ophthalmology	CB NEI
Other:	Carl Kupfer M.D.	Director	NEI
	Lessie McCain R.N.	Clinical Technician	CB NEI
	Manuel Datiles M.D.	Visiting Scientist	CB NEI

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Branch

SECTION

Ophthalmic Genetics and Pediatric Ophthalmology

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.80	0.30	0.50

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to compare patients having pigment dispersion syndrome with and without glaucoma. The data acquired may enable a determination of the risk of patients with pigment dispersion syndrome to develop glaucoma as well as add to understanding of the pathology of the disease.

Project Description:

Protocol Number: 76 EI 189

Objectives: To compare patients having pigment dispersion with and without glaucoma by documenting and following the clinical features and course of their disease and by evaluating the patient's performance on a variety of diagnostic tests. To determine the presence of abnormal aqueous humor dynamics using provocative testing in patients having pigmentary dispersion with and without glaucoma. To compare pigment dispersion with and without glaucoma with respect to possible genetic markers. To determine whether pupillary responses to light stimulation are abnormal in cases having iris transillumination.

Methods Employed: At the first visit, the following examinations are performed:

Complete family history with detailed pedigree
Best corrected visual acuity with manifest refraction
Slit lamp examination
Visual field examination (Goldmann I_{2e} and I_{4e})
Applanation Goldmann tension (app)
Photography of iris transillumination
Goniophotography

At the next visit, the following examinations are performed:

Static perimetry
Base-line tonography and water-drinking tonography one hour later
Fasting blood sugar when indicated

At the third visit, the following examinations are performed:

Slit lamp photography of Krukenberg spindle
Dilated ophthalmoscopic examination (two and one-half percent phenylephrine and one percent cyclogel)
Stereophotographs of the optic nervehead

At the fourth visit, pupillography is performed.

Major Findings: Patients may have pigment dispersion syndrome for as long as 20 years without developing glaucoma.

There may be a hereditary predisposition in some cases, as seen in a mother and daughter, two brothers and one son, and a brother and sister.

Steroid testing and PTC taste testing do not appear to show any particular categorization of these patients. Recent evidence has indicated that HLA antigens in patients with pigment dispersion are also not significantly different than those in the normal population.

Project No. Z01 EY 00011-08 CB

It may be noted that whether filtering procedures are performed or not, pigment may be lost from the trabecular meshwork in time.

More than 80 patients are currently enrolled in the study. Four patients have been demonstrated to have unilateral involvement. Two patients have very marked evidence of PDS with deep anterior chambers, marked transillumination and heavy pigment in the angle structures. However, neither have developed elevated intraocular pressure.

Significance to Biomedical Research and the Program of the Institute: These data may enable a determination to be made of the risk of patients having pigment dispersion to develop glaucoma. Specifically, it may be possible to identify which features of these determinations have predictive value in forecasting which of those patients having pigment dispersion will develop a visual field defect. In addition, the relationship of "pigmentary" glaucoma to the known characteristics of open-angle glaucoma can be investigated.

Proposed Course: This project will be continued for three more years to continue to obtain data to further understand the knowledge about pigment dispersion syndrome.

NEI Research Program: Glaucoma--Developmental, Congenital, or Infantile Glaucoma

Publications: None



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00060-06 CB								
PERIOD COVERED October 1, 1981, to September 30, 1982											
TITLE OF PROJECT (80 characters or less) Visual Function and Ocular Pigmentation in Albinism											
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT											
<table> <tr> <td>PI:</td> <td>Muriel I. Kaiser-Kupfer M.D.</td> <td>Chief, Section on Ophthalmic Genetics and Pediatric Ophthalmology</td> <td>CB NEI</td> </tr> <tr> <td>Other:</td> <td>Lessie McCain Rafael Caruso</td> <td>R.N. Clinical Technician M.D. Expert</td> <td>CB NEI CB NEI</td> </tr> </table>				PI:	Muriel I. Kaiser-Kupfer M.D.	Chief, Section on Ophthalmic Genetics and Pediatric Ophthalmology	CB NEI	Other:	Lessie McCain Rafael Caruso	R.N. Clinical Technician M.D. Expert	CB NEI CB NEI
PI:	Muriel I. Kaiser-Kupfer M.D.	Chief, Section on Ophthalmic Genetics and Pediatric Ophthalmology	CB NEI								
Other:	Lessie McCain Rafael Caruso	R.N. Clinical Technician M.D. Expert	CB NEI CB NEI								
COOPERATING UNITS (if any)											
LAB/BRANCH Clinical Branch											
SECTION Ophthalmic Genetics and Pediatric Ophthalmology											
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205											
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:									
0.12	.04	.08									
CHECK APPROPRIATE BOX(ES)											
<input checked="" type="checkbox"/> (a) HUMAN SUBJECTS		<input type="checkbox"/> (b) HUMAN TISSUES									
<input type="checkbox"/> (c) NEITHER											
<input type="checkbox"/> (a1) MINDRS		<input type="checkbox"/> (a2) INTERVIEWS									
SUMMARY OF WORK (200 words or less - underline keywords)											
<p>Patients with hypomelanotic disorders such as ocular albinism, oculocutaneous albinism, Chediak-Higashi Disease, Hermansky-Pudlak Syndrome and iris trans-illumination defects are being recruited to determine visual function and to evaluate its course over time. Family members are evaluated to attempt to determine factors which may identify the heterozygous state.</p>											

Project Description:

Protocol Number: 76 EI 207

Objectives: The objectives of the study are to relate the level of visual function to the amount of ocular pigmentation, especially iris and retinal pigmentation; to correlate the amount of nystagmus with visual acuity and iris pigmentation; to determine whether ocular pigmentation, visual acuity, and nystagmus change with age; and to identify the heterozygous state in family members.

Methods Employed: The following examinations are performed:

Complete family history with detailed pedigree
Best corrected visual acuity at near and distance with refraction
Slit lamp examination
Psychophysical testing including D-15 and Munsell 100 hue, rod and cone thresholds
Dilated ophthalmoscopic examination
Hair bulb incubation
Photography to document hair color, eye color, iris transillumination, disc, and macula
Visual evoked response

Examination of family members includes:

Best corrected visual acuity
Slit lamp examination of iris
Photography of iris transillumination
Fundus examination when vision not corrected to 20/20

Major Findings: Examination of patients and family members indicates that the finding of transillumination of the iris may be seen in the absence of recognized albinism. The pattern appears to be punctate and may be present in a diffuse manner or limited to the 6 o'clock sector.

Significance to Biomedical Research and the Program of the Institute: These data may allow identification of the carrier state in albinism which would be of importance in genetic counselling. In addition, it may be possible to determine whether the development of the fovea is abnormal in albinism, and if this is the cause of the decreased visual acuity in albinism or whether decreased visual acuity is secondary to hypopigmentation and the resultant light-scatter and glare. In addition, it will be possible to ascertain whether visual acuity improves with age and if this is correlated with changes in pigmentation.

Proposed Course: This project will be continued for five more years in order to obtain additional data.

NEI Research Program: Retinal and Choroidal Diseases--Developmental and Hereditary Disorders

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00062-06 CB																
PERIOD COVERED October 1, 1981, to September 30, 1982																			
TITLE OF PROJECT (80 characters or less) Progressive Essential Iris Atrophy																			
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																			
<table> <tr> <td>PI:</td> <td>Muriel I. Kaiser-Kupfer M.D.</td> <td>Chief, Section on Ophthalmic Genetics and Pediatric Ophthalmology</td> <td>CB NEI</td> </tr> <tr> <td>Other:</td> <td>Carl Kupfer M.D.</td> <td>Director</td> <td>NEI</td> </tr> <tr> <td></td> <td>Lessie McCain R.N.</td> <td>Clinical Technician</td> <td>CB NEI</td> </tr> <tr> <td></td> <td>Manuel Datiles M.D.</td> <td>Visiting Scientist</td> <td>CB NEI</td> </tr> </table>				PI:	Muriel I. Kaiser-Kupfer M.D.	Chief, Section on Ophthalmic Genetics and Pediatric Ophthalmology	CB NEI	Other:	Carl Kupfer M.D.	Director	NEI		Lessie McCain R.N.	Clinical Technician	CB NEI		Manuel Datiles M.D.	Visiting Scientist	CB NEI
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COOPERATING UNITS (if any)																			
LAB/BRANCH Clinical Branch																			
SECTION Ophthalmic Genetics and Pediatric Ophthalmology																			
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205																			
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:																	
0.5	0.2	0.3																	
CHECK APPROPRIATE BOX(ES)																			
<input checked="" type="checkbox"/> (a) HUMAN SUBJECTS		<input type="checkbox"/> (b) HUMAN TISSUES																	
		<input type="checkbox"/> (c) NEITHER																	
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																			
SUMMARY OF WORK (200 words or less - underline keywords)																			
<p>Patients are being recruited with progressive essential iris atrophy with or without associated corneal disease. Information is being gathered to evaluate the clinical features and course of the disease process, to investigate aqueous humor dynamics in both affected and unaffected eyes, and to attempt to find genetic markers such as HLA and ABO antigens or physical correlates with the disease process.</p>																			

Project Description:

Protocol Number: 76 EI 219

Objectives: The objectives of the study are to develop a panel of patients with progressive essential iris atrophy and to study these patients to determine factors which may aid in understanding the pathophysiology of the disease process and to study the natural history of this disease. Measurements of aqueous humor dynamics, assessment of genetic markers such as HLA and ABO antigens and physical correlates, and iris fluorescein angiography to determine the role of the vasculature will be carried out.

Methods Employed: During the course of the evaluation, the following procedures are performed:

Complete family history with detailed pedigree
Best corrected visual acuity with manifest refraction
Slit lamp examination
Visual field examination (Goldmann I_{2e} and I_{4e})
Photography of iris and iris transillumination
Specular microscopy
Gonioscopy and goniophotography
Iris fluorescein angiography and photography
Baseline tonography
A complete medical and dental evaluation
Dilated ophthalmoscopic examination
Stereophotographs of the optic nervehead

Major Findings: Histopathologic and electron microscopic study of iris and trabecular meshwork tissue has not indicated any clues to the pathogenesis of the disease process.

An ultrathin corneal contact lens is useful in certain patients to prevent recurrent rupture of corneal bullae.

Six patients have been recruited into the study.

Although the eye with essential atrophy presents the major problem in management with glaucoma and corneal abnormalities our studies show subclinical involvement of the second eye with changes of either decreased outflow facility, iris transillumination and corneal endothelial pathology.

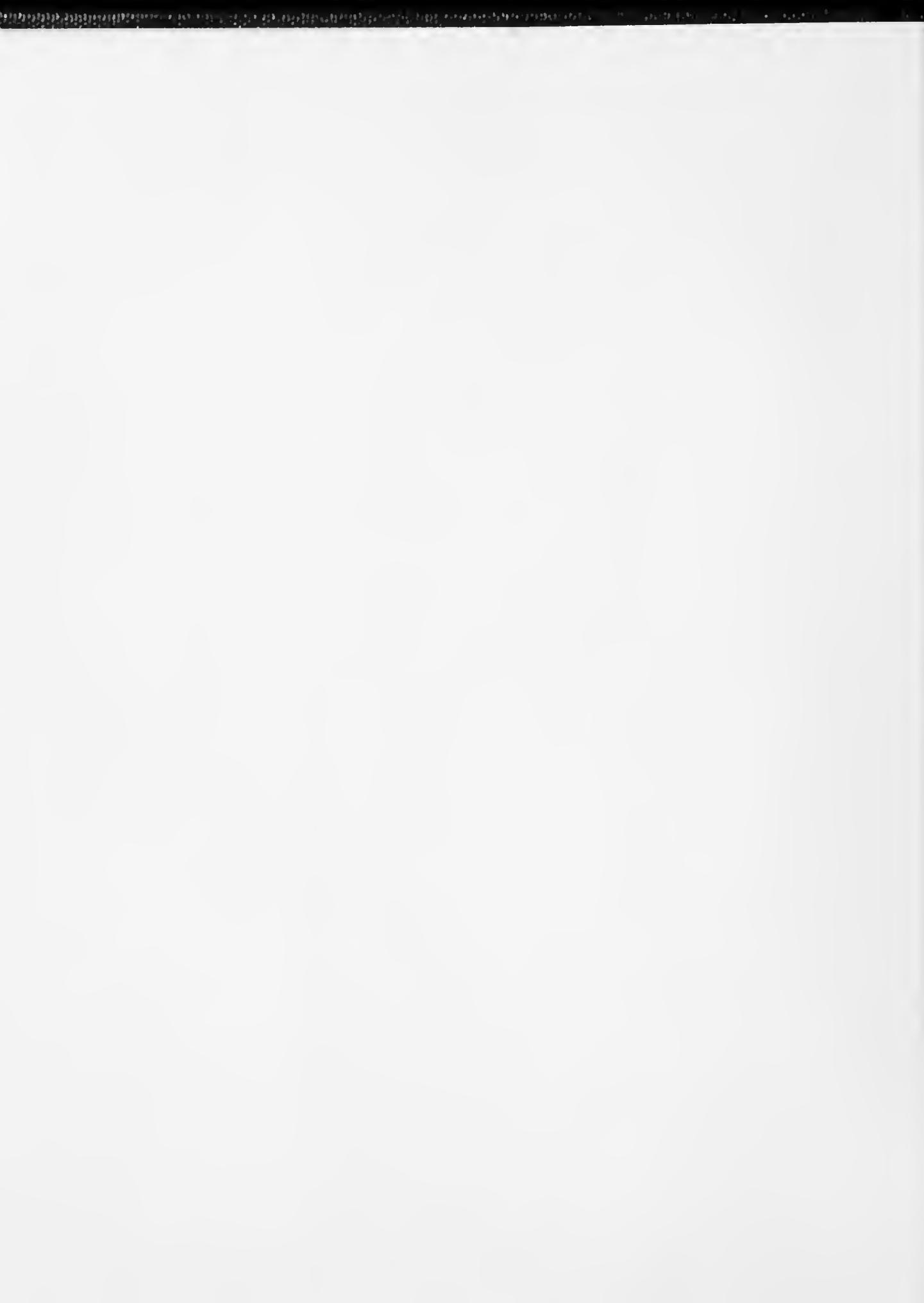
Significance to Biomedical Research and the Program of the Institute: These data may contribute to an understanding of pathophysiologic factors involved in the rare entity of progressive essential iris atrophy. In addition, a careful study of the progression of the disease from the earliest signs will clarify the significance of corneal involvement and the status of outflow channels which may add to the understanding of the mechanism of glaucoma.

Project No. Z01 EY 00062-06 CB

Proposed Course: The project will continue for four more years in an effort to obtain more data regarding the pathophysiology of this process.

NEI Research Program: Glaucoma--Developmental, Congenital, or Infantile Glaucoma

Publications: None



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00083-05 CB
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PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

The Diagnosis, Pathogenesis and Treatment of Gyrate Atrophy of the Choroid and Retina

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Muriel I. Kaiser-Kupfer M.D.	Chief, Section on Ophthalmic Genetics and Pediatric Ophthalmology	CB NEI
Other:	Francisco de Monasterio M.D.	Chief, Section of Visual Processing	CB NEI
	Manuel Datiles M.D.	Visiting Scientist	CB NEI

COOPERATING UNITS (if any)

Department of Pediatrics and Medicine, The Johns Hopkins University School of Medicine, Baltimore, Maryland

LAB/BRANCH

Clinical Branch

SECTION

Ophthalmic Genetics and Pediatric Ophthalmology

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.50	0.50

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Patients with gyrate atrophy of the choroid and retina are examined systematically to confirm the diagnosis. Skin fibroblasts of affected patients and family members grown in tissue culture are assayed for ornithine amino- δ -transferase activity. The results will be examined for correlation with the presence of homo- or heterozygosity for the disease trait. Patients will be given a trial of pyridoxine to see if serum concentration of ornithine can be reduced, and if so, the patient will be classified as a "responder," and treatment with pyridoxine will be continued. Nonresponder and responder patients will be placed on a low arginine, low protein diet with supplemental amino acids and observed for an arrest or improvement of their disease.

Project Description:

Protocol Number: 78 EI 01

Objectives: To determine the biochemical processes responsible for the elevated serum ornithine and the chorioretinal lesion that occurs in gyrate atrophy of the retina. To determine which patients respond to pyridoxine treatment with a decrease in serum ornithine concentration. To determine if treatment of "responders" with pyridoxine and/or dietary manipulation will arrest the progress of the retinal atrophy.

Methods Employed: Patients suspected of having gyrate atrophy of the retina are examined according to a standard set of procedures to confirm the diagnosis. Plasma ornithine concentration is measured periodically. Punch biopsies of the skin are grown in tissue culture, and their enzymatic activity related to ornithine metabolism is measured.

Major Findings: Patients with gyrate atrophy of the retina have been shown to have a deficiency of ornithine- δ -aminotransferase. A small percentage of patients with gyrate atrophy have a 30-50 percent decrease of serum ornithine while on pyridoxine therapy. One patient in this study has been followed for 41 months on a low protein, low arginine diet and was found to show an improvement in dark adaptation averaged ERG and color vision testing after 13.5 months on this regime with lowered plasma ornithine levels. A second patient after 15 months with lowered plasma ornithine levels showed improved visual fields and color vision testing. Ten additional patients have been placed on the diet. All sustained a significant reduction of plasma ornithine while in the hospital. However, following discharge one patient discontinued the diet, five showed poor control, one showed fair control and three showed good control.

Systemic findings in this condition have been documented and confirmed which include abnormalities of hair, EEG abnormalities and tubular aggregates in muscle.

Significance to Biomedical Research and the Program of the Institute: Gyrate atrophy of the retina is the first of the genetically determined isolated severe retinal degenerations for which a specific biochemical marker and concomitant enzyme defect has been demonstrated. The study will guide and test the efficacy of treatment for this blinding eye disease and serve as a model for the investigation of other genetically determined retinal degenerations.

Proposed Course: This project will be continued for three more years to further assess the knowledge of reduced ornithine in halting the chorio-retinal degeneration.

NEI Research Program: Retinal and Choroidal Diseases--Developmental and Hereditary Disorders

Project No. Z01 EY 00083-05 CB

Publications:

Valle D, Kaiser-Kupfer MI: Gyrate Atrophy of the choroid and retina
Clinical, Structural, and Biochemical Advances in Hereditary Eye
Disorders. New York, Alan R. Liss, Inc., 1982, pp 123-134.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00084-04 CB

PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (30 characters or less)

Anterior Chamber Anomalies Associated with Glaucoma or Ocular Hypertension

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Carl Kupfer	M.D.	Director	NEI
Other: Muriel I. Kaiser-Kupfer	M.D.	Chief, Section on Ophthalmic Genetics and Pediatric Ophthalmology	CB NEI
Lessie McCain	R.N.	Clinical Technician	CB NEI
Manuel Datiles	M.D.	Visiting Scientist	CB NEI

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Branch

SECTION

Ophthalmic Genetics and Pediatric Ophthalmology

INSTITUTE AND LOCATION

National Eye Institution, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.30	0.10	0.20

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS

(b) HUMAN TISSUES

(c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

With recent embryological research indicating the role of the neural crest in contributing to all connective tissues anterior to the lens epithelium, the group of developmental anomalies of the anterior chamber with glaucoma or ocular hypertension are being reviewed.

Project Description:

Protocol Number: 77 EY 119

Objectives: The objective of this study is to determine whether congenital and/or developmental anomalies of the anterior chamber are related to faulty migration or terminal differentiation of neural crest tissue.

Methods Employed: Patients of all ages with congenital and/or developmental anomalies of the anterior chamber are being examined clinically to determine involvement of cornea, trabecular meshwork, iris stroma, lens and ciliary body. When intractable glaucoma is present that cannot be controlled with medication, surgery will be performed and the specimens examined histologically.

Major Findings: It appears that in this group of anomalies of anterior chamber development there are pathological changes in one or several tissues derived from neural crest. These include corneal stroma, corneal endothelium, anterior iris stroma, Descemet's membrane, and trabecular meshwork endothelium.

Significance to Biomedical Research and the Program of the Institute:
A better understanding of the pathogenesis of these glaucomas may help in improving diagnosis and treatment.

Proposed Course: Patients with other anomalies of the anterior chamber including congenital cataracts will be examined for abnormalities in tissue derived from neural crests.

NEI Research Program: Glaucoma--(Developmental, Congenital, or Infantile Glaucoma)

Publications:

Kaiser-Kupfer MI, White BJ (By Invitation), Papadopoulos N, (By Invitation): Aniridia and Mental Retardation with Deletion of the Short Arm. Trans Am Ophthalmol Soc LXXIX: 1981.

Kupfer C, Datiles M, Kaiser-Kupfer MI: Development of the Anterior Chamber of the Eye: Embryology and Clinical Implications. Medizin und Naturwissenschaften (in press)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00075-04 CB
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PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Immune Functions in Ocular Diseases of Obscure Etiology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Robert Nussenblatt	M.D.	Chief, Ophthalmic Immunology Section	CB	NEI
Other: Igal Gery	Ph.D.	Visiting Scientist	LVR	NEI
William Leake	M.S.	Biologist	CB	NEI

COOPERATING UNITS (if any)

Department of Ophthalmology, University of Louisville, Louisville, Kentucky
Wilmer Eye Institute, Johns Hopkins Hospital, Baltimore, Maryland

LAB/BRANCH
Clinical Branch

SECTION
Section on Ophthalmic Immunology

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.0 PROFESSIONAL: 0.2 OTHER: 0.8

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In vitro cellular immune functions are being studied in a masked method in patients with ocular toxoplasmosis, pars planitis, Behcet's disease, ocular sarcoid, birdshot choroidopathy, geographic choroiditis and chorio- retinitis of unknown origin. Crude ocular antigens as well as the purified uveitogenic soluble antigen (S-antigen) of the retina are being used in a lymphocyte microculture technique in order to evaluate the presence of cellular immune memory to ocular tissues. Immune memory is also evaluated by the production of lymphokine in a capillary migration system. A subgroup of patients with posterior uveitis has been identified as having this immunologic memory. Other studies concentrate on the presence of suppressor cell activity functioning of macrophages and lymphocyte subsets as defined by monoclonal antibodies in these patients. These results shed light on the basic mechanisms of uveitis and may be used as a guide for specific immunologic therapy.

Project Description:

Protocol Number: 79 EI 49

Objectives: The objective of this study is to investigate several immunological factors in ocular inflammatory disease and how they may relate to the course and chronicity of this disease. The identification of groups with specific immunologic alterations provide us with a more rational approach to therapy.

Methods Employed: The ophthalmic examination of all patients includes slit lamp examination, visual field tests, electroretinogram, and fluorescein angiography. Lymphocyte cultures are prepared using the microculture technique, where the immune cells are tested against various crude ocular extracts, as well as purified human bovine S-antigen, in order to assess evidence of cellular immune memory which is considered to be the *in vitro* equivalent of the anamnestic response *in vivo*. The capillary migration system is used to evaluate migration inhibition of macrophages, a test considered as an *in vitro* equivalent of lymphokine production *in vivo*. Suppressor cells from patients during latent and active ocular disease are induced in the laboratory by using concanavalin A, with their suppression capabilities tested *in vitro* in the presence of fresh responder cells and mitogens. Suppressor cell activity is also evaluated by the use of suboptimal doses of concanavalin A in culture, as reported by Bresnihan and Jasin (J Clin Invest 59:109, 1977). Macrophage activity is studied by examining their production of lymphocyte activating factor. Monoclonal antibodies to T cell subsets, in conjunction with the fluorescein activated cell sorter, are, in addition, being used in an attempt to identify alterations in lymphocyte subgroups.

Major Findings: A subpopulation of patients with ocular inflammatory disease manifested a positive "memory" response to the S-antigen. Positive responders appear to be those with active or inactive retinal lesions, and patients with various diseases were found to respond. It therefore appears that similar immune groups are present in different clinical entities.

Some patients with posterior uveitis respond to crude retinal extracts but not to the S-antigen, indicating the possible role of other retinal antigens still to be purified. All patients with Birdshot retinochoroidopathy that were tested manifest cell-mediated responses to either the 5-Ag rv crude retinal antigens.

Posterior uveitis patients manifested increased Con A induced suppression when compared to controls. But these same patients had decreased suppression when measured by the method described by Bresnihan and Jasin.

Significance to Biomedical Research and the Program of the Institute: Uveitis is the cause of five percent of legal blindness in the United States. This is the first time that patients' immune cells have been shown to manifest cellular immune memory to a purified retinal antigen, and that alterations in suppressor cells are also present.

The grouping of patients with uveitis on the basis of specific immunologic functions or alterations may provide a more rational basis upon which to develop specific immunotherapy. Elucidation and treatment of inflammatory conditions of the eye are major interests of the NEI.

Proposed Course: This continuing study will focus on the posterior uveitic entities in order to investigate further the role of the S-antigen in each of these, and what, if any role abnormal suppressor cell activity may play.

NEI Research Program: Retinal and Choroidal Diseases--Inflammatory Disorders

Publications:

Nussenblatt RB, Mittal KK, Ryan S, Green WR, Maumenee AE: Birdshot retinochoroidopathy - an association with HLA-A29 and immune responsiveness to retinal S-antigen. Am J Ophthalmol (in press).



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00092-04 CB
PERIOD COVERED October 1, 1981, to September 30, 1982			
TITLE OF PROJECT (80 characters or less) HLA, ABO, and B-cell Alloantigens and Ocular Inflammatory Disease			
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT			
PI: Robert Nussenblatt M.D. Chief, Ophthalmic Immunology CB NEI Section			
Other: None			
COOPERATING UNITS (if any) Bureau of Biologics, FDA			
LAB/BRANCH Clinical Branch			
SECTION Section on Ophthalmic Immunology			
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205			
TOTAL MANYEARS: 0.4	PROFESSIONAL: 0.1	OTHER: 0.3	
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS			
SUMMARY OF WORK (200 words or less - underline keywords) <p>Patients with <u>ocular toxoplasmosis</u>, <u>pars planitis</u>, <u>sarcoidosis</u>, <u>Behcet's disease</u>, <u>chorioretinitis of unknown origin</u>, and <u>birdshot choroidopathy</u> are being studied to determine the phenotype frequency of the <u>HLA</u>, <u>ABO</u>, and <u>B-cell alloantigens</u>. Since the <u>B-cell alloantigens</u> or <u>DR antigens</u> are thought to play a role in the <u>immunologic response to antigens</u>, these findings will complement other immune uveitis studies being simultaneously carried out.</p>			

Project Description:

Protocol Number: 79 EI 48

Objectives: To determine whether patients with ocular inflammatory disease manifests specific HLA or B-cell alloantigens more frequently than the average population.

Methods Employed: Heparinized blood samples from patients are subjected to microcytotoxic tests to determine the HLA and B-cell antigens. The ABO system is evaluated utilizing an anti-sera method.

Major Findings: HLA-B8 has been found to be associated with iridocyclitis in black Americans. This antigen has been associated with a wide range of autoimmune diseases, and its presence in patients with this disorder strongly suggests a similar mechanism for this disease. 80% of the birdshot retinochoroidopathy patients tested were positive for HLA-A29. The $p < .0001$, with the computed relative risk also 50, one of the highest recorded.

Significance to Biomedical Research and the Program of the Institute: The role of HLA and B-cell alloantigens in the immune response is only beginning to unfold. This study will indicate whether these alloantigens play a role in the ocular immune response.

Proposed Course: This study will continue in order that sizeable populations of various ocular immune entities will be studied.

NEI Research Program: Retinal and Choroidal Diseases--Inflammatory Disorders

Publications:

Nussenblatt, RB., Mittal, KK., Ryan S, Green WR, Maumenee AE: Birdshot retinochoroidopathy - an association with HLA-A29 and immune responsiveness to retinal S-antigen. Am J Ophthalmol (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00093-04 CB
PERIOD COVERED October 1, 1981, to September 30, 1982			
TITLE OF PROJECT (80 characters or less) Cataracts in Juvenile Guinea Pigs with Allergic Encephalomyelitis			
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT			
PI:	Robert Nussenblatt M.D.	Chief, Ophthalmic Immunology Section	CB NEI
Other:	Sanford Stone M.D.	Head, Immunology Unit	OSD NIAID
COOPERATING UNITS (if any) Department of Pathology, Albert Einstein College of Medicine Office of the Scientific Director, NIAID			
LAB/BRANCH Clinical Branch			
SECTION Section on Ophthalmic Immunology			
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205			
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:	
0.9	0.3	0.6	
CHECK APPROPRIATE BOX(ES)			
<input type="checkbox"/> (a) HUMAN SUBJECTS		<input type="checkbox"/> (b) HUMAN TISSUES	
<input checked="" type="checkbox"/> (c) NEITHER			
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS			
SUMMARY OF WORK (200 words or less - underline keywords)			
<p>Allergic encephalomyelitis is a central nervous system disease of immunologic origin. In juvenile strain 13 guinea pigs, cataracts developed during severe allergic (autoimmune) encephalomyelitis syndromes produced actively or by transfer of living lymph node cells from sensitized strain 13 donors. These lens changes were manifested bilaterally within a two-week period of active sensitization or transfer of sensitized cells. The morphologic in vivo appearance of these cataracts is similar to both the galactosemic induced and tryptophan deficiency cataract models. A better understanding of the etiology of these lesions not seen before in this entity in guinea pigs will help in understanding cataract formation in systemic disease.</p>			

Project Description:

Objectives: To investigate the etiology of cataract formation in young inbred animals which develop an acute autoimmune neurologic disease.

Methods Employed: Induction of allergic encephalomyelitis in juvenile strain 13 guinea pigs is accomplished in one of two ways. The first method for immunization of these animals is the injection of guinea pig spinal cord in complete Freund's adjuvant into multiple nuchal sites. A second method is the induction of the disease in strain 13 adults or juveniles with the subsequent transfer of immunologically active cells to the histocompatible juvenile animals.

Each animal is observed carefully for evidence of weight loss, urinary incontinence, hind-limb wasting, and cataracts.

Major Findings: The majority of juvenile strain 13 animals which were recipients of transfers of lymph node cells from histocompatible juvenile or adult donors showed bilateral cataracts. A large number of those actively immunized also manifested the same lesions. The opacities are first located in the cortex and have a doughnut appearance, with fully opacified lenses being the end result. These lesions did not appear in nonhistocompatible guinea pig recipients. Initial biochemical analysis indicates the presence of unusual soluble lens proteins.

Significance to Biomedical Research and the Program of the Institute: Cataract formation in guinea pigs has never been reported before with induction of this well-known immunologic model. This cataract model could provide an understanding of how systemic diseases may alter the ocular environment so as to induce lenticular opacities.

Proposed Course: We will study the biochemical basis for the lens changes and attempt to prevent the induction of these cataracts during the disease.

NEI Research Program: Retinal and Choroidal Diseases--Retinal Detachment and Vitreous Disorders

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00094-04 CB
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PERIOD COVERED

October 1, 1981, to September 30, 1982
TITLE OF PROJECT (80 characters or less)

Immune Mechanisms in Experimental Autoimmune Uveitis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert Nussenblatt	M.D.	Chief, Ophthalmic Immunology Section	CB	NEI
Other:	Igal Gery	Ph.D.	Visiting Scientist	LVR	NEI
	Mario Salinas-Carmona	M.D., Ph.D.	Visiting Fellow	CB	NEI
	Merlyn Rodrigues	M.D.	Chief, Section on Clinical Eye Pathology	CB	NEI
	William Leake	M.S.	Biologist	CB	NEI

COOPERATING UNITS (if any)

Department of Ophthalmology, University of Louisville, Kentucky

LAB/BRANCH

Clinical Branch

SECTION

Section on Ophthalmic Immunology

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0.0

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Guinea pig strain 13 animals, Lewis rats, and non-human primates immunized at a site distant to the eye with the Soluble antigen (S-antigen) of the retina in complete Freund's adjuvant develop experimental allergic uveitis (EAU). Depending on the antigen immunizing dose and the animal, the ocular lesions can vary from an iridocyclitis to a panuveitis. Lymph node cells, nonadherent T-cells obtained from peritoneal exudate cells, and peripheral lymphocytes from immunized animals manifested significant cellular immune responses whether measured by the lymphocyte culturing technique or by evidence of the production of migration inhibition factor (MIF) of macrophages. Ocular electrophysiologic (ERG) alterations seen in non-human primates with S-antigen uveitis are similar to those seen in patients with posterior uveitis. Cyclosporin A, a drug with specific anti-T-cell activity, has been found to be exceptionally effective

Project Description:

Objectives: We have previously reported that experimental uveitis may be induced in animals by immunization with a purified component of the retina (S-antigen). This study is designed to elucidate the basic immunologic mechanisms of this laboratory model for uveitis and how this model may be altered or regulated.

Methods Employed: Strain 13 guinea pigs, Lewis rats, and non-human primates are immunized with purified S-antigen in complete Freund's adjuvant in one hind footpad or the nuchal region. Evidence of ocular inflammatory disease is monitored via slit lamp and ophthalmoscopic examinations. After two to four weeks, lymph node, peritoneal exudates, or peripheral blood cells are collected and used for several cellular immune studies. Lymphocyte cultures are prepared in microtiter plates and are stimulated with S-antigen as well as other antigens. Other immune cells from immunized animals are mixed with isogeneic macrophages in order to demonstrate the release of migration inhibition factor in the presence of S-antigen. Lewis rats immunized with the S-antigen are "protected" by daily injections of Cyclosporin A. Antibodies are evaluated by gel diffusion, ELISA, and indirect hemagglutination techniques, and eyes are taken for histology.

Major Findings: Animals immunized with S-antigen develop obvious clinical anterior and posterior uveitis which is confirmed by histology. Animals with ocular disease manifest significant cellular immune memory responses when measured by lymphoproliferative and macrophage inhibition techniques.

The EAU model in non-human primates parallels closely the disease seen in some posterior uveitis patients. EAU could not be induced in the homozygote nude rat. However, it could be in the heterozygote, and T-cells from these rats transferred to the nude rat yielded ocular inflammatory disease in 5-7 days. The finding that CsA therapy inhibited the development of the S-Ag induced uveitis adds insight into mechanisms. In addition, anti-S-Ag antibody titers were observed to be similar in rats protected and not protected with CsA. Knowing CsA's anti-T-cell effects, this study would support the need for T-cell participation in EAU.

Significance to Biomedical Research and the Program of the Institute: Experimental autoimmune uveitis is the first uveitis model utilizing a purified retinal antigen. The mapping out of its immune mechanisms may lead to an improved understanding of human ocular inflammatory disease. Immunoregulatory models developed in this system will be utilized in future human clinical trials, including Cyclosporin A.

Proposed Course: To describe fully the underlying immune events in this disease and to develop a successful protocol dealing with either specific or nonspecific suppression of the disease.

NEI Research Program: Retinal and Choroidal Diseases--Inflammatory Disorders

Publications:

Salinas-Carmona MS, Nussenblatt RB, Gery I: Experimental autoimmune uveitis in the athymic nude rat. Eur J Immunology (in press).

Nussenblatt RB, Rodrigues MM, Salinas-Carmona MC, Gery I, Cevario S, Wacker, W: Modulation of experimental autoimmune uveitis with Cycloporin A. Arch Ophthalmol 100:1146-1149, 1982.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00115-02 CB
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PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Cyclosporin A Therapy in Uveitis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert B. Nussenblatt	M.D.	Chief, Ophthalmic Immunology Section	CB	NEI
Other:	Francisco de Monasterio	M.D., D.Sc.	Chief, Section on Visual Processing	CB	NEI
	Kent E. Higgins	Ph.D	Senior Staff Fellow	CB	NEI
	Igal Gery	Ph.D.	Visiting Scientist	LVR	NEI
	Alan Palestine	M.D.	Senior Staff Fellow	CB	NEI
	Chi Chan	M.D.	Senior Staff Fellow	CB	NEI
	William Leake	M.S.	Biologist	CB	NEI

COOPERATING UNITS (if any)

Department of Immunology, National Naval Medical Center, Bethesda, Maryland

LAB/BRANCH

Clinical Branch

SECTION

Section on Ophthalmic Immunology

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.4	0.6

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Cyclosporin A, a endecapeptide product with specific anti-T-cell characteristics, will be administered to patients with sight threatening ocular inflammatory disease of non-infectious origin. This will be done in order to test Cyclosporin's efficacy in the treatment of uveitis.

Project Description:

Protocol Number: 81-EI-33

Objectives: Cyclosporin A (CsA), an endecapeptide obtained from fungi, has been shown to have specific anti-T-cell activity (Transplantation Proc. 12:234, 1980). We have reported CsA's exceptional effectiveness in preventing the induction of S-antigen autoimmune uveitis in rats, as well as the inhibition of the disease once immunization has occurred (J Clin Invest 67:1228, 1981). The goal of this study will be to test CsA's efficacy in treating patients with bilateral sight threatening posterior uveitis of an autoimmune nature.

Methods Employed: Ten patients will be initially selected in this pilot project. Patients twenty-one years of age or older, of either sex (females not pregnant), will be admitted to this study. All patients should have a bilateral sight threatening uveitis of non-infectious etiology. Lymphocyte cultures are prepared where the immune cells are tested against various crude ocular extracts, as well as purified human S-antigen, in order to assess evidence of cellular immune memory which is considered to be the in vitro equivalent of the anamnestic response in vivo. Patients chosen will be treated with CsA for six months. During this period, the patients' clinical, immunologic, and ocular electrophysiologic course will be closely monitored.

Major Findings: This study is still recruiting patients and no results can be given at this time.

Significance to Biomedical Research and the Program of the Institute: Uveitis is one of the most frustrating problems in all of ophthalmology. Present modes of therapy for patients with severe ocular inflammatory disease are inadequate, non-specific, and have a myriad of side effects. CsA, if effective, will be an important adjunct to therapy, and will, by virtue of its known immune properties, add to our knowledge of mechanisms in this disease.

Proposed Course: Studies of Cyclosporin A Therapy in Uveitis will be continued.

NEI Research Program: Retinal and Choroidal Diseases—Inflammatory Disorders.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00116-02 CB

PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Double Masked Treatment of Ocular Toxoplasmosis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert B. Nussenblatt	M.D.	Chief, Ophthalmic Immunology Section	CB	NEI
Other:	Francisco de Monasterio	M.D., D.Sc.	Chief, Section on Visual Processing	CB	NEI
	Daniel Seigel	D.Sc.	Deputy Chief	OBE	NEI
	Igal Gery	Ph.D.	Visiting Scientist	LVR	NEI
	Marvin Podgor	M.S.	Statistician	OBE	NEI
	Chi Chan	M.D.	Senior Staff Fellow	CB	NEI

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Branch

SECTION

Section on Ophthalmic Immunology

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of the project is to evaluate whether clindamycin combined with sulfadiazine will prove as or more effective therapy for ocular toxoplasmosis than the combination of sulfadiazine and daraprim. Patients with active toxoplasmosis will be randomized within strata (determined by size of lesion and proximity to the macula) to one of the two treatments, in this double masked study.

Project Description:

Protocol Number: 81-EI-92

Objectives: Ocular toxoplasmosis represents a sizable number of cases seen in an uveitis clinic. Sulfadiazine and daraprim have been considered the combination of choice in the therapy of sight-threatening toxoplasma lesions. Reports have now suggested that clindamycin may be effective therapy for toxoplasmosis. The objective of this study is to randomize patients in a double masked study in order to compare the efficacy of sulfadiazine/clindamycin and sulfadiazine/daraprim therapy.

Methods Employed: Patients 18 years or older, of either sex (females not pregnant), will be admitted to this study. They should manifest an active retinal lesion due to toxoplasmosis. Patients will receive a standard ophthalmic examination and will be randomized into a therapy group on the basis of the size and position of the active lesion. The cause of the disease will be followed clinically, as well as with electrophysiologic testing. The data is to be collected by the OBE-NEI and evaluated.

Major Findings: This study does not have enough participants in order to report any significant findings.

Significance to Biomedical Research and the Program of the Institute: Toxoplasmosis is the cause of a large number of uveitis cases in the United States. Daraprim has potentially serious side effects. If another form of therapy can be demonstrated equally or more effective than sulfadiazine and daraprim, then clinicians will be given an expanded choice in dealing with this potentially sight threatening problem.

Proposed Course: Studies on Double Masked Treatment of Ocular Toxoplasmosis will be continued.

NEI Research Program: Retinal and Choroidal Diseases--Inflammatory Disorders.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00107-03 CB

PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Suppression of Retinoblastoma Proliferation by Human Soluble Lymphocyte Factors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Mario Salinas-Carmona	M.D.	Visiting Fellow	CB	NEI
Other:	Robert Nussenblatt	M.D.	Chief, Ophthalmic Immunology Section	CB	NEI
	Paul Russell	Ph.D.	Research Chemist	LVR	NEI
	John Hooks	Ph.D.	Research Microbiologist	LOM	NIDR

COOPERATING UNITS (if any)

Laboratory of Oral Medicine, NIDR

LAB/BRANCH

Clinical Branch

SECTION

Section on Ophthalmic Immunology

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:
1.0

PROFESSIONAL:
0.25

OTHER:
0.75

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS

(b) HUMAN TISSUES

(c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Mononuclear cells when stimulated with concanavalin A develop different biological activities, including suppressor activity. The objective of this work is to find out whether the inhibitory activity is mediated through soluble factors, and to characterize these factors' biological and physiochemical properties. We have found that the factors responsible for suppression are non-dialyzable, heat stable, resistant to pH2 treatment and inhibits proliferation of a variety of cells including human lymphocytes, retinoblastoma cells and stromal keratocytes.

Project Description:

Objectives: Human mononuclear cells when stimulated with concanavalin A (Con A) develop different biological activities. Under specific culture conditions, and in the presence of that mitogen, some lymphocytes inhibit proliferation of fresh autologous or allogeneic lymphocytes. The mechanism by which those cells exert their suppressor activity is not known. The objectives of the present work is to investigate whether the inhibitory effect of Con A activated human lymphocytes is mediated through soluble factors, and if so, characterize their biological and physiochemical properties.

Methods Employed: Purified lymphocyte populations were stimulated with Con A for different periods of time. The resultant cell supernatants were sterilized by membrane filtration and tested against fresh allogeneic lymphocytes, retinoblastoma cells, and stromal keratocytes. Tritiated methyl thymidine uptake is used to assess cell proliferation. Biochemical methods such as membrane ultrafiltration, sieve chromatography, pH2 and enzyme treatment of crude supernatants as well as the semi-purified fractions were performed to determine some properties of the suppressor factors; bioassays such as interferon determinations were also done.

Major Findings: Concanavalin A (Con A) or phytohemagglutinin activate a population of human circulating lymphocytes to exert suppressive functions. We found that supernates from the activated human lymphocytes suppress lymphocyte responses to Con A, the mixed lymphocyte reaction and pokeweed mitogen-induced IgM production. Mitogen stimulated suppressor lymphocytes, or their supernates, inhibit also the spontaneous proliferation of human retinoblastoma cells (Y-79 line) and primary cultures of human keratocytes. A correlation was always noted between the levels of inhibitory activities of the lymphocytes and their supernates. Furthermore, a good correlation was found between the levels of inhibition by the supernates of lymphocyte functions (proliferation and IgM production) and of the nonlymphoid cells' proliferation. Some of the properties of this suppressor factor(s) are (i) produced only by the T-cell population; (ii) appears after 8 hrs of Con A stimulation, peaks at 24 to 48 hrs and declines later on; (iii) stable at 56°C and labile at 70°C; (iv) non-dialyzable and present in the 40K-100K dalton fraction of a G-200 Sephadex column; (v) labile to pH 2 treatment.

Significance to Biomedical Research and the Program of the Institute: A purified suppressor factor from normal human mononuclear cells has been sought for some time. Its identification is of great benefit in understanding basic mechanisms of immuno-regulation.

Proposed Course: This project has been terminated.

NEI Research Program: Retinal and Choroidal Diseases--Inflammatory Disorders

Publications:

Salinas Carmona MC, Gery I, Russell P, Nussenblatt, RB: Mitogen induced suppressor factor(s) from human lymphocytes. Cellular Immunol 71:44-53, 1982.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00135-10 CB
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PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Biochemistry of Retina and Pigmented Epithelium in Health and Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Helen H. Hess,	M.D. Medical Officer	CB NEI
Other: David A. Newsome	M.D. Chief, Section on Retinal and Ocular Connective Tissue Diseases	CB NEI
Gloria E. Westney	B.S. Biologist	CB NEI
Joseph J. Knapka	Ph.D. Nutritionist, Small Animal Section	VRB DRS
Ira Levine	B.S. Guest Worker	CB NEI

COOPERATING UNITS (if any)

Veterinary Resources Branch, DRS, NIH

LAB/BRANCH

Clinical Branch

SECTION

Section on Retinal and Ocular Connective Tissue Diseases

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.8	1.3	0.5

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Investigations are being conducted into the biochemical composition of the sensory retina, pigmented epithelium, choroid and biological fluids in normal and disease states, particularly in animal models of human retinal degenerations and in human retinal diseases. The tissue specific distributions of inorganic constituents are studied by flameless atomic absorption, with concentrations of Cu, Zn and Ca of particular interest. These elements are being assayed in 24 hour urine specimens from patients with retinal degenerations (retinitis pigmentosa and macular degenerations). The effects of nutrition and genetic background on the progress of chorioretinal degeneration in the retinal dystrophic pigmented RCS rat are being investigated. Age of onset and incidence of posterior subcapsular cataracts and the history of progression of mature cataracts are being studied in both pink-eyed and black-eyed retinal dystrophic RCS rat models of retinal degeneration-related cataract. Nutritional and environmental factors involved in incidence and prevention of the cataracts are being investigated.

Project Description:

Objectives: To study the biochemical composition of retinal photoreceptor, neuronal, glial, pigmented epithelial cells and biological fluid in health and disease, and to explore possibilities for prevention or therapy of disease of the retina and/or pigmented epithelium-choroid when a biochemical abnormality has been identified. This exploration extends to possibilities of prevention or therapy of cataracts which often accompany retinal and/or pigmented epithelium-choroid diseases. Diseases in which the retinal pigmented epithelium (RPE) is involved are of particular interest.

Methods Employed: Defined diets are prepared and fed to affected and congenic unaffected retinal dystrophic animals in controlled experiments. Clinical findings are recorded after indirect ophthalmoscopic and biomicroscopic examination and fundus photography, slitlamp examination and slitlamp photography. Analytical methods include flameless atomic absorption spectrophotometry, light and electron microscopy, enzymatic assays by spectrophotometry and fluorometry, and standard quantitative biochemical determinations as appropriate. Twenty-four hour urine samples from humans with retinal disease are examined for trace metal content.

Major Findings:

I. Slitlamp Assessment of Age of Onset and Incidence of Cataracts in Pink-eyed, Tan-hooded Retinal Dystrophic RCS Rats:

Posterior subcapsular cataracts (PSC) are associated with hereditary retinal dystrophy in the Royal College of Surgeons (RCS) rat model and with human retinitis pigmentosa. The relationship of lens and retinal pathology has never been explained. Previous studies of pink-eyed RCS rats aged 2.5-11 months had shown an incidence of cataract of 24 percent when observed by the unaided eye and 60 percent by direct ophthalmoscopy, while 40 percent of rats were considered to have clear lenses. Unlike the retinal degeneration, which appeared in all homozygous animals, cataract seemed not to be predictably associated with the rdy mutation. To test this further, we studied the lenses of rats of different ages with a diagnostic slitlamp. We confirmed that by 8 to 15 months of age, rats fed a diet containing recommended concentrations of all known nutrients for rodents (NIH natural ingredient diet) developed cataracts with an incidence of 23 percent when observed by unaided eye. In addition, opacities were seen in 74 percent with the indirect ophthalmoscope and 20 D lens; but 100 percent had at least a "sugar grain" type PSC by slitlamp. The slitlamp-detectable cataract was first seen in some animals at 49 days, and by 56 days all rats examined had bilateral PSC. This is an age at which the rod photoreceptors have degenerated. We concluded that slitlamp-detectable PSC are predictably associated with the retinal dystrophy of the rdy mutation. The RCS rat model may be relevant to a type of human retinal degeneration having a constant association of cataract.

II. Slitlamp Assessment of Onset of Cataracts in Black-eyed, Black-hooded Retinal Dystrophic RCS Rats:

Having shown that all pink-eyed retinal dystrophic RCS rats examined had bilateral slitlamp-detectable PSC with an onset at 7-8 weeks of postnatal age, we then studied the congenic black-eyed retinal dystrophic RCS rats similarly. The black-eyed dystrophic rat may be a better model than the pink-eyed rat for some type of hereditary retinal degeneration in humans, most of whom have pigmented eyes. The black-eyed rats were shown previously to have a much lower incidence of mature cataracts (3 percent) by 2.5 to 11 months of age (LaVail et al., 1975), but the age of onset was not studied. Black eye pigmentation also has been shown to have a marked retarding effect on the rate of retinal degeneration, consisting of 10 days in the posterior retina and 35 days in the peripheral part of the superior hemisphere, but no effect in the peripheral part of the inferior hemisphere. These effects are the same as those produced by dark rearing of pink-eyed dystrophics (LaVail and Battelle, 1975). We applied the sensitive diagnostic slitlamp technique to determine whether the black-eyed dystrophic had an early onset of cataractous change or whether occurrence of PSC was delayed in parallel with the delays in rates of retinal degeneration in the different retinal regions or the delay in onset of the mature cataracts. We found that the slitlamp-detectable PSC began at the same time in the black-eyed as in the pink-eyed dystrophics, namely at 7 to 8 weeks of postnatal age. Eye pigmentation appears, therefore, not to delay onset of PSC. The RCS rat may have more direct relevance as a model for human PSC than for human retinal degeneration, since the specific human counterpart of the rat retinal disease has not yet been identified.

III. Nutritional Effects on Manifestation of Cataracts in Pink-eyed and Black-eyed Retinal Dystrophic RCS Rats:

We noted previously that pink-eyed RCS rats fed a sunflower kernel-supplemented closed formula diet over several generations failed to develop any mature cataracts in adulthood, although such cataracts had been seen in 23 percent of pink-eyed dystrophics fed the NIH natural ingredient diet. After demonstrating that both pink- and black-eyed RCS rats had an onset of slitlamp-detectable PSC at 8 weeks of postnatal age, we examined adult pink-eyed dystrophic rats fed the sunflower kernel supplemented closed formula diet. In a group of 28 pink-eyed RCS rats ranging in age from 7 to 23 months, slitlamp-detectable PSC were observed in less than 2 percent of eyes. Furthermore, 15 black-eyed RCS between the ages of 8 and 15 months failed to show slitlamp-detectable PSC and the mature cataracts failed to develop in the pink and black-eyed dystrophics fed the sunflower kernel supplemented closed formula diet. Controlled experiments have therefore been undertaken, including the use of defined-composition diets, to attempt to elucidate the relative importance of nutritional factors in the sunflower kernel supplemented diet that prevent occurrence of cataracts in the hereditarily disposed RCS rat, and to look for nutritional factors in the NIH natural ingredient diet that may be

involved in permitting the cataracts to be manifested. The various diets are fed first to a parental generation, then to a an F 1 parental generation and to their F 2 offspring, which represent the experimental group. Sixty pink-eyed and 110 black-eyed dystrophic rats are studied with each diet. The work is being carried out with the collaboration of Dr. Joseph J. Knapka, nutritionist, DRS. Cataracts are also being examined by transillumination in vitro, and by histopathologic techniques. Retinas are being studied to look for any changes in rate of degeneration with the different dietary regimens. Biochemical experiments using standard techniques are being conducted on retina, pigmented epithelium and lens, to attempt to assess differences in chemical pathology.

IV. Studies of Lysosomal Enzymes in Black-eyed Retinal Dystrophic RCS Rats and Their Congenic Controls:

Lysosomal enzyme activities were assayed in neural retinas and pigmented epithelium-choroid tissues of age-matched pigmented dystrophic RCS rats and congenic pigmented RCS controls. Fluorescence methods using substrates of 4-methyl-umbelliferol were adapted to determine Bhexosaminidase (degrades glycoproteins and glycolipids), B-glucuronidase (degrades proteoglycans) and a-glucosidase (degrades glycogen of lysosomes). Animal ages varied from 10 to 240 days. Results were similar between dystrophics and unaffecteds. In the RPE-choroid both B-hex and B-gluc decreased 4-5 fold and a-gluc about 8-fold between days 10 and 30. Enzyme activities were then constant through 240 days except for B-hex, which showed a 3.5 fold rise in both groups between 120 and 180 days, which persisted through 240 days. Acid hydrolase activities were lower in retinas than in fellow RPE-choroids. Highest enzyme activities in retina were at 10 days, and then remained stable through 240 days. These data show no outstanding correlation between hydrolase activities and retinal dystrophy in the black-eyed RCS rat, but reveal an interesting age-dependent variation in enzyme activities which will bear further study. Further work will seek to determine whether the enzyme activities are predominantly associated with RPE or with choroid, and to extend the age range to 1-9 postnatal days. At the younger ages, RPE can be isolated from the choroid readily, and attempts are being made to do this at older ages. RPE cell cultures can be established and biochemical assays conducted on these for comparison with results on fresh tissues.

V. Mineral Metabolism in Human Retinal Disease:

The trace elements copper and zinc are being studied by flameless atomic absorption spectrophotometric assay of 24 hour urine specimens from patients with retinitis pigmentosa and macular degenerations, including angioid streaks.

Copper: Interest in the 24 hour urinary excretion of copper by patients with retinal degenerations was aroused by a report of Gahlot et al. (1976) that in patients with primary retinitis pigmentosa (RP) the rate was six-fold that in normal subjects. Two subsequent studies from Denmark and the US failed to support this finding in RP patients, although a second independent report from

India (Rao et al., 1981) continued to support the idea that some RP patients excrete more copper in a 24 hour period. Because of these controversial reports and the previous clinical observation that a family with vitelliform retinal degeneration had several other members with hepatolenticular degeneration (Brink, 1974) we lengthened our series of patients from whom 24 hour urines were collected. Using flameless atomic absorption, we have not confirmed the increased excretion of copper, in a series of 52 patients with retinitis pigmentosa and 40 members of their families. In this series, only one RP urine had a value for copper (60 ug/24 hr) that lay outside the normal range of 0-30 ug/24 hour. All values in 41 controls fell within that range except one (48 ug/24 hour). Values for 24 hr excretion of copper also were within the normal range in 87 patients with macular degenerations and in 41 of their close relatives. Several possibilities for explanation of the high values in RP patients in India relative to the normal values in patients from other countries include: (1) a genetic isolate of RP patients with high Cu excretion may exist in India; (2) some aspect of diet may be involved in higher Cu excretion in the Indian patients; or (3) a technical problem may exist in comparing the data obtained by colorimetric and atomic absorption methods.

Zinc: We have been determining Zn in the same urines in which Cu has been done. This survey now includes more than 300 patients and controls. The normal values for both males and females agree well with those in the recent literature. Replicate samples of urine have been obtained at different times on some patients, with similar results. It will be necessary to go beyond the current type of experimental design to try to investigate what appear to be abnormal Zn excretion tendencies in certain families. The population group includes retinitis pigmentosa of various types, as well as drusenoid macular degeneration, juvenile hereditary macular degeneration, fundus flavimaculatus, and idiopathic angiod streak, which is a special type of macular degeneration. Additional familial cases of the different types of pigmentary retinal degeneration showing increased or decreased 24 hour Zn excretion will be recruited to complete the series.

These studies illustrate that as compared with sporadic cases, family groups have greater potential for revealing possibly significant findings with an economy of laboratory and clinical effort. They also show the importance of accumulating a relatively large series of control and diseased specimens before publishing the results.

Significance to Biomedical Research and the Program of the Institute: Retinal deteriorations are the major cause of untreatable blindness in the United States and probably in the world. The retinal pigmented epithelium is becoming increasingly appreciated as the primary site of many of these disease processes. Posterior subcapsular cataracts occur in various types of hereditary retinal degenerations, in humans and in RCS rats, as well as in older persons and in some persons treated with steroids. Nutritional and genetic factors are thought to play key roles in many human diseases, and can often be studied in detail in animal models, giving an opportunity to develop ways to prevent or cure the diseases. Information gained from these studies should contribute to our understanding of human disease and to initiating and conducting trials of possible therapeutic measures.

Project No. Z01 EY 00135-10 CB

Proposed Course: The project will be continued with emphasis on controlled trials of nutritional regimens and rigorous elucidation of nutritional variables in retinal dystrophic animals. Human urine specimens will be analyzed from some additional familial groups.

NEI Research Program: Retinal and Choroidal Diseases--Developmental and Hereditary Disorders

Publications:

Hess HH, Newsome DA, Knapka JJ, Bieri JG: Effects of sunflower seed supplements on reproduction and growth of RCS rats with hereditary retinal dystrophy. Lab Anim Sci 31:482-488, 1981.

Hess HH, Newsome DA, Knapka JJ and Westney GE: Sunflower kernel supplemented diet and decreased incidence of cataracts in RCS rats with hereditary retinal dystrophy. Invest Ophthalmol Vis Sci 22(3, Suppl): 285, 1982.

Levine I, Hess HH and Newsome DA: Lysosomal activities vary with age in pigmented RCS rats and congenic controls. Invest Ophthalmol Vis Sci 22(3, Suppl):260, 1982.

Hess HH, Newsome DA, Knapka JJ and Westney GE: Slit lamp assessment of age of onset and incidence of cataracts in pink-eyed, tan-hooded retinal dystrophic rats. Curr Eye Res (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00057-04 CB

PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Ocular Connective Tissue Macromolecules and Their Function in Vision

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Pamela Gehron Robey	Ph.D.	Staff Fellow	CB	NEI
Other:	David A. Newsome	M.D.	Chief, Section on Retinal and Ocular Connective Tissue Diseases	CB	NEI
	Judy A. Kirshner	B.S.	Biological Aide	CB	NEI

COOPERATING UNITS (if any)

Laboratory of Developmental Biology and Anomalies, NIDR

LAB/BRANCH

Clinical Branch

SECTION

Section on Retinal and Ocular Connective Tissue Diseases

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.50	1.25	0.25

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS

(b) HUMAN TISSUES

(c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Extra cellular matrix components, such as collagen, proteoglycans and other glycoconjugates are being identified in ocular tissues such as trabecular meshwork, sclera, lens capsule and chorioretinal complex, with particular emphasis on Bruch's membrane. These components are isolated from the tissues by various extraction procedures and compared to the components synthesized by organ culture of these tissues in the presence of radiolabeled precursors. Biochemical characterization is accomplished by column chromatography, sodium dodecyl sulfate polyacrylamide gel electrophoresis and enzymatic and chemical degradations. Alterations of the connective tissue components may play a role in certain ocular diseases such as in glaucoma and in drusenoid macular degeneration. Diseased tissues are also being studied to gain insight into the role of extracellular matrix components in these disorders.

Project Description:

Objectives: The extracellular matrix of connective tissues consists of an orderly network of collagen fibers, proteoglycans and glycoproteins. The presence, interaction, and arrangement of these structural macromolecules is crucial to the normal function of these tissues, the outflow rate of the trabecular meshwork and filtration through Bruch's membrane. The purposes of this study include characterization of the collagens, proteoglycans, and glycoproteins normally present in the sclera, trabecular meshwork, choroid and Bruch's membrane, and the determination of the alterations that occur in these macromolecules in certain ocular diseases.

Methods Employed: Ocular connective tissue samples are radiolabeled in organ culture or cells derived from these tissues are grown and labeled in cell culture. The naturally occurring macromolecules are also extracted and characterized. Biosynthetically labeled as well as unlabeled matrix components are characterized using molecular sieve chromatography, DEAE-cellulose chromatography, CMC-cellulose chromatography, immunoprecipitation, gel electrophoresis, cesium chloride density gradient centrifugation, as well as with specific enzymes, such as collagenase, chondroitinase, keratanase, glycosidases, papain, and pepsin.

Major Findings: Trabecular meshwork dissected from Rhesus monkeys and placed in organ culture synthesize a large amount of hyaluronic acid as well as a chondroitin/dermatan sulfate. However, when the entire anterior segment is placed in organ culture and the trabecular meshwork is dissected after labeling, the proportions of the various components are different. Relative to hyaluronic acid, there are increased amounts of newly synthesized glycoproteins and proteoglycans present. The system developed here will be used to study the components in normal and glaucomatous trabecular meshwork tissue.

The proteoglycan components of Bruch's membrane have not been well characterized to date. The glycosaminoglycans have now been identified and were found to be heparan sulfate with small amounts of chondroitin and/or dermatan sulfate and hyaluronic acid. The biosynthesis of glycosaminoglycans and their incorporation into proteoglycans was investigated using an eye organ culture in which the cornea, iris and sclera had been removed. The newly synthesized proteoglycan(s) extracted with 4M guanidine hydrochloride bound to DEAE-cellulose and eluted with approximately 0.4M NaCl, and were found to have a molecular weight of between 100,000 and 150,000 daltons. After papain treatment, the glycosaminoglycan side chains had a molecular weight of approximately 44,000 daltons. The newly synthesized proteoglycan(s) contained 65% chondroitin and/or dermatan sulfate and 35% heparan sulfate. This organ culture system should be useful in studying disease states of Bruch's membrane.

Significance to Biomedical Research and the Program of the Institute: Connective tissue is by far the predominant tissue of the eye. It is likely that alterations in the quantity or quality of the macromolecules which comprise these tissues will be the basis of certain blinding and visually disabling ocular diseases.

Proposed Course: This study may provide information that will allow the formulation of testable therapeutic modalities. The project will continue by utilizing appropriate animal models and human material. Antibodies to purified collagen and glycoconjugates will be prepared for use in clinical and biomedical research.

NEI Research Program: Corneal Diseases--Corneal Dystrophies, Inherited Disorders, and Developmental Anomalies

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00065-05 CB
PERIOD COVERED October 1, 1981, to September 30, 1982			
TITLE OF PROJECT (80 characters or less) Physiological Studies of the Visual System of Primates			
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT			
PI:	Francisco M. de Monasterio M.D., D.Sc.	Chief, Section on Visual Processing	CB NEI
Other:	Stanley J. Schein M.D., Ph.D.	Expert	CB NEI
	Edna P. McCrane B.S.	Biologist	CB NEI
	Kent E. Higgins Ph.D.	Senior Staff Fellow	CB NEI
	Robert Desimone Ph.D.	Staff Fellow	LN NIMH
COOPERATING UNITS (if any)			
LAB/BRANCH Clinical Branch			
SECTION Section on Visual Processing			
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205			
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:	
3.5	3.5	0.0	
CHECK APPROPRIATE BOX(ES)			
<input type="checkbox"/> (a) HUMAN SUBJECTS		<input type="checkbox"/> (b) HUMAN TISSUES	
<input checked="" type="checkbox"/> (c) NEITHER			
<input type="checkbox"/> (a1) MINORS		<input type="checkbox"/> (a2) INTERVIEWS	
SUMMARY OF WORK (200 words or less - underline keywords)			
<p>This project is a study of the physiological organization of neurons in the visual system of non-human primates that may serve as a model for the human visual system. The project gives emphasis to the <u>chromatic</u> and <u>spatial</u> properties and <u>central projections</u> of neurons of the <u>retina</u>, <u>lateral geniculate body</u>, <u>striate cortex</u> and <u>extrastriate cortex</u> of macaque monkeys.</p>			

Project Description:

Objectives: To study the neural organization underlying the processing of visual data in retina to cortex, with particular emphasis on color vision.

Methods Employed: Intracellular and extracellular recordings from single neurons, intracellular staining with fluorescent dyes, and extracellular recordings of mass responses.

Major Findings:

I. Extracellular recordings from neurons of the extrastriate cortex of Old World macaque monkeys. (Schein, Desimone, de Monasterio).

Recordings were made from neurons located within the central representation of the V4 area of extrastriate visual cortex using a semi-chronic, nitrous oxide preparation; the properties of the cells were examined in enough detail to permit their classification in terms of spatial and color properties. Cyto-architectural and myeloarchitectural studies of the cortical region confirmed the identification of the area where the recordings were made.

Broad-band (Wratten filters) color stimuli of matched photopic luminosity, showed that nearly all cells responded to white. We adopted a criterion that if a response to any one color was more than twice as great as any other, the cell was classified as color-biased. About half of the cells were color-biased, half were not. Narrow band (interference filters) color stimuli of matched photopic luminosity, allowed a similar conclusion.

Narrow-band color stimuli, matched for equal quanta, demonstrated that some cells showed an underlying color-opponency in the hand-mapped receptive field, even though the cells responded well to white. "Silent" surrounds, in which a stimulus could not produce a response, were found to affect the response to a stimulus located within the hand-mapped receptive field, the center. These silent surrounds were frequently of the opposite chromatic preference.

These findings are significant in two respects. First, the presence of silent surrounds, which appeared to be a feature of most V4 neurons, is an important advance in image processing by the brain over that reported in V1, primary visual cortex. Second, we are beginning to be able to reconcile conflicting reports on the chromatic preference of V4 neurons.

II. Extracellular recordings from retinal ganglion cells of Old-World macaque monkeys.

A. Studies of spectral response bandwidths. (de Monasterio, Schein)

The spectral response bandwidth of color-opponent retinal ganglion cells were examined in conditions of neutral adaptation. Color-opponent cells show specific "signatures" in plots of response bandwidth vs. the wavelength of the peak sensitivity that allow for an acceptable estimate of the type(s) of cone input mediating cell responses.

Averaged spectral bandwidths of color-opponent ganglion cells were compared with published data from neurons of subsequent levels of the geniculocortical pathway, including the extrastriate area termed V4. No significant differences were found between color selective cells of the retina, dorsal lateral geniculate body, striate cortex and V4, which on the average have a half-bandwidth of 25 nm at half maximum sensitivity.

The spectral location of the peak sensitivity of responses of the various types of color-opponent ganglion cells shows a comparatively broad distribution, which loosely clusters at some spectral loci. Comparison of this distribution with that reported in several studies of V4 cell responses indicates a nearly complete absence of 'blue-yellow' opponent responses in those cortical studies. In association with more recent electrophysiological studies of V4 cells, carried out by ourselves and other workers, the results of the present study do not support current claims of a color-specialization of this extrastriate cortical area.

B. Organization of the peripheral 'blue-yellow' pathway of macaques.
(de Monasterio and Higgins)

We have examined the spectral and spatial properties of a specific group of color-opponent ganglion cells of the macaque retina. These cells, termed 'blue-yellow', have opponent responses mediated, on the one hand, by blue cone input and, on the other hand, by combined input from red and green cones. These cells show a relatively small incidence (ca. 15 percent) and three major varieties can be distinguished: (i) 'blue-center, yellow surround', (ii) 'yellow-center, bluesurround' and (iii) cells in which the opponent 'blue' and 'yellow' responses have a coextensive distribution over the receptive field. 'Blue-center' cells show two subvarieties: small-field cells, whose receptive-field center is as small as that of other classes of color-opponent cells, and large-field cells, whose centers are larger. Small-field cells predominate in the foveal region while large-field predominate in the extrafoveal region.

'Blue-center' cells show a most pronounced ON-center bias (90 percent of the blue-center' cells), which is less obvious and probably absent in other varieties. This blue-ON bias has not been found, so far, in the striate cortex, and current cortical data fail to support its existence.

Responses mediated by signals from blue cones show transient loss of sensitivity at both the onset and the offset of long-wavelength backgrounds which, per se, should produce negligible quantum catches in the blue cone themselves. These transients, therefore, indicate color opponent interactions between signals from blue cones and from red and/or green cones. The transients are likely to be generated prior to the level of the ganglion cells, probably at the outer plexiform layer, and they closely resemble transient adaptational anomalies observed in psychophysical studies using similar stimuli.

While 'blue-yellow' opponent cells have been found in the lateral geniculate body but not the superior colliculus, suggesting that they project to the cerebral cortex via the geniculo-striate radiation, current data from the striate cortex have not yet provided unambiguous evidence of the existence of these cells.

III. Intracellular recordings in the isolate monkey retina. (de Monasterio)

We continue the development of a preparation based on the isolated, superfused monkey retina which would allow for the electrophysiological study of single cell properties and the anatomical connections of retinal cells. Whereas we have been able to maintain rod-mediated responses over several hours, cone-mediated responses remain labile. Present emphasis is on modifications of the composition of the perfusing medium that may permit more resilient cone-mediated responses.

Significance to Biomedical Research and the Program of the Institute:
Understanding the organization of the visual system of non-human primates is most valuable for understanding the mechanisms of visual processing of the human visual system, which at present can only be studied by indirect methods.

Proposed Course: Both extracellular and intracellular recordings from single cells of the monkey visual system will be continued.

NEI Research Program: Strabismus, Amblyopia, and Visual Processing--
Ocular Motility and Strabisms (Structure and Function)

Publications:

de Monasterio FM, Schein SJ: Absence of spectral bandwidth narrowing from retina to area V4. Neurosci Abstr 7:832, 1981.

Schein SJ, Marrocco RT, de Monasterio FM: Is there a high concentration of color-selective cells in the V4 area of monkey visual cortex?
J Neurophysiol 47:193-213, 1982.

de Monasterio FM, Schein SJ: Spectral bandwidths of color-opponent cells of the geniculo-cortical pathway of macaque monkeys. J Neurophysiol 47:214-224, 1982.

de Monasterio FM, Higgins KE: Organization of the peripheral 'blue-yellow' opponent pathway of macaque monkey. J Neurophysiol (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00059-04 CB

PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Electrophysiological and Psychophysical Evaluation of Retinal Disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Francisco M. de Monasterio	M.D., D.Sc.	Chief, Section on Visual Processing	CB	NEI
Other:	Kent E. Higgins	Ph.D.	Senior Staff Fellow	CB	NEI
	Rafael C. Caruso	M.D.	Expert	CB	NEI
	Ralph D. Gunkel	O.D.	Ophthalmic Physicist	CB	NEI
	Myles J. Jaffe	O.D.	Guest Worker	CB	NEI
	Stanley J. Schein	M.D., Ph.D.	Expert	CB	NEI
	Doris Collie	A.A.	Health Technician	CB	NEI
	Mary Fuhrman		Health Technician	CB	NEI
	Patricia Mercer	B.S.	Health Technician	CB	NEI

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Branch

SECTION

Section on Visual Processing

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

6.75

PROFESSIONAL:

6.75

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS

(b) HUMAN TISSUES

(c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This is a general support and service-providing project for the inflammatory, degenerative, or congenital retinal disorders, and to conduct tests and experiments directed towards the clinical application and development of electrophysiological and psychophysical procedures for measuring visual function in patients of NEI's Eye Clinic and of other services in the NIH Clinical Center.

Project Description:

Objectives: Diagnosis or evaluation of visual function in toxic, inflammatory, degenerative, and congenital visual disorders affecting the retina. Development of clinical procedures for the study of visual function.

Methods Employed: Commercially available and laboratory-developed instruments are used in measuring visual function in normal volunteers and clinical patients on the basis of electroretinography (single flash and averaged Ganzfeld, averaged Focal), visually evoked cortical potentials, electroculography, spatial contrast sensitivity, sensory rod and cone thresholds, color-vision testing, retinal image stabilization, visual perimetry and other psychophysical functions.

Major Findings: A battery of different electrodiagnostic, electrophysiological and psychophysical tests were performed in 2109 inpatients, outpatients and referred consult cases for purposes of collaborative studies with other members of the medical staff of the Clinical Branch.

Present efforts continue to be directed towards the development of new tests in color vision and of a non-invasive system of retinal image stabilization for clinical procedures which would allow for studies of focal electroretinography with very small stimuli at different retinal eccentricities and locations, microperimetry and psychophysical functions.

Significance to Biomedical Research and the Program of the Institute: Development of new research techniques and the application of new and existing research techniques to clinical procedures are expected to help improve the diagnosis of visual disorders and the understanding of physiopathological mechanisms of retinal disease.

Proposed Course: Electrophysiological and psychophysical studies of retinal disorders will be continued.

NEI Research Program: Retinal and Choroidal Diseases--Noninvasive Techniques in the Study of Retinal Disorders

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00061-04 CB

PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Retinal Function in Posterior Uveitis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Francisco M. de Monasterio	M.D., D.Sc.	Chief, Section on Visual Processing	CB	NEI
Other:	Kent E Higgins	Ph.D.	Senior Staff Fellow	CB	NEI
	Stanley J. Schein	M.D., Ph.D.	Expert	CB	NEI
	Myles J. Jaffe	O.D.	Guest Worker	CB	NEI
	Patricia Mercer	B.S.	Health Technician	CB	NEI
	Doris Collie		Health Technician	CB	NEI
	Mary Fuhrman		Health Technician	CB	NEI

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Branch

SECTION

Section on Visual Processing

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

2.7

PROFESSIONAL:

2.7

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

 (a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER (a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Abnormalities of retinal function at the level of rods and cones or their pathways are being documented by electrophysiological and psychophysical studies of patients with posterior uveitis of suspected immunological origin, in addition to experimental studies of posterior uveitis in animal models.

Project Description:

Protocol Number: 79 EI 49

Objectives: To understand the retinal physiopathology of posterior-segment uveitis and chorio-retinitis of suspected immunological origin.

Methods Employed: Retinal function is assessed by electroretinography (single flash and averaged Ganzfeld responses, focal responses), electrocucography, sensory dark-adaptation thresholds, visual perimetry, color vision tests and contrast sensitivity functions in cases of ocular toxoplasmosis, pars planitis, Behcet's disease, ocular sarcoid, Vogt-Kayanagi-Harada's syndrome, ocular histoplasmosis and other inflammatory diseases affecting the posterior segment of the eye.

Major Findings: Studies of patients with inflammation of the posterior segment indicate that diffuse and central involvement of the retina produces early electroretinographic waveform changes of cone responses and color vision deficiencies. The electroretinogram changes primarily involving responses mediated by signals from red- and green-sensitive cones, are accompanied by reduction or extinction of responses mediated by signals from blue-sensitive cones. These alterations, which appear to be an accurate diagnostic criteria to detect inflammatory activity of immune origin, are accompanied by relatively typical, though unspecific, tritan or tetartan-like defects of central vision. We are at present examining specific losses in spatial contrast sensitivity.

The observed changes in electroretinography, coupled with color vision alterations and other psychophysical findings represent a nearly pathognomonic sign of central retinal involvement which seems to be associated with cell-mediated responses to the retinal S-antigen. These results have been confirmed in electrophysiological studies of S-antigen induced posterior uveitis in Old-World monkeys.

Present emphasis is given to more/detailed psychophysical studies, especially spatial contrast sensitivity functions of these patients. We are at present also attempting to determine by the use of fluorescent dyes alterations in the density and morphology of blue-sensitive cones in experimental uveitis.

Significance to Biomedical Research and the Program of the Institute: The detected electrophysiological signs serve to study the clinical evolution of the cases with diffuse central uveitis with retinal involvement using comparatively simple tests. Characterization and localization of disordered retinal function may elucidate some of the physiopathological processes of immunological retinal disease.

Proposed Course: Studies of retinal function in posterior uveitis will be continued.

NEI Research Program: Retinal and Choroidal Diseases--Inflammatory Disorders

Project No. Z01 EY 00061-04 CB

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00063-04 CB

PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Acquired and Congenital Color Vision Deficiencies: Mechanisms and
DiagnosisNAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER
PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Francisco M. de Monasterio	M.D., D.Sc.	Chief, Section on Visual Processing	CB	NEI
Other:	Kent E. Higgins	Ph.D.	Senior Staff Fellow	CB	NEI
	Stanley J. Schein	M.D., Ph.D.	Expert	CB	NEI
	Rafael C. Caruso	M.D.	Expert	CB	NEI
	Myles J. Jaffe	O.D.	Guest Worker	CB	NEI
	Marvin J. Podgor	M.S.	Statistician	OBE	NEI
	Edna P. McCrane	B.S.	Biologist	CB	NEI
	Patricia Mercer	B.S.	Health Technician	CB	NEI
	Doris Collie		Health Technician	CB	NEI
	Mary Fuhrman		Health Technician	CB	NEI
	J. Kelly Newlander		Summer Student	CB	NEI

COOPERATING UNITS (if any)

Lion's Eye Bank, Washington, D.C.

LAB/BRANCH

Clinical Branch

SECTION

Section on Visual Processing

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

6.0

PROFESSIONAL:

6.0

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

 (a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER (a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is a study of cone function in selected cases of color vision defects, with special emphasis on acquired defects. Human subjects are examined with electrophysiological and psychophysical tests, while experimental studies are carried out in subhuman primates.

Project Description:

Protocol Number: 79 E1 92

Objectives: To characterize and document color vision abnormalities mediated by dysfunction of blue-sensitive, red-sensitive, and green-sensitive cones, or of their retinal pathways.

Methods Employed: Color vision is examined on the basis of a battery of psychophysical tests (increment thresholds, field and test spectral sensitivity of pi-mechanisms, spectral luminosity, chromagraph and saturation discrimination tests), electrophysiological studies of cone responses. Cytochrome oxidase reaction for human eyes.

Major Findings:

I. KÖLLNER'S Rule (de Monasterio, Schein, Caruso, McCrane).

Early observations by H. KÖLLNER indicated that whereas most types of retinal disease result in an acquired "blue-yellow" defect in color vision, disease of the optic nerve commonly leads to a "red-green" defect. KÖLLNER's rule is interpreted today as indicating a particular vulnerability of blue-cone function to retinal insult. We have obtained evidence that the introduction of fluorescent dyes to the eye of Old-World macaque monkeys results in the specific staining of blue-sensitive cones. Our understanding of the mechanism of the complete staining of the cell body of these cones, accompanied by the lack of staining of other cones and rods, provided experimental support for the clinical observations of KÖLLNER. The staining of blue cones of monkey retina could be explained by their lower threshold to toxic insults. In the case of the dyes we used, they altered membrane function of blue cones, but not of other photoreceptors, and this dysfunction resulted in cell death and the consequent penetration of the dye into the blue cones. No specific blue-cone staining is obtained when dyes that do not affect membrane function to a significant degree were injected intravitreally.

Based on these techniques we are also attempting to develop a sub-human private model for acquired "blue-yellow" defects due to retinal insult. This model should provide an experimental tool to help improving clinical electrodiagnostic techniques.

II. Staining of cones of pathological retinae (Schein, de Monasterio, McCrane).

Cytochrome oxidase, a mitochondrial enzyme, is particularly rich in the ellipsoid of cone outer segment. In brain, cytochrome oxidase reaction may be used to assess chronic activity levels. We have reacted retinas of enucleated eyes of human donors and obtained "control" patterns of reaction, showing relatively uniform staining of all cones. We plan to apply the technique to eyes from humans with documented color vision defects of the "loss" type in order to obtain a non-uniform pattern of reaction, providing identification of the dysfunctional cone. In protanopia, it might be possible to identify the red-cone mosaic in this fashion.

III. Human blue-sensitive cone function and retinal aging (de Monasterio, Jaffe, Podgor).

We have measured the peak amplitude and implicit time of ERG b-waves mediated respectively by signals from dark-adapted rods, dark-adapted blue cones and dark-adapted red/green cones in male and female normal volunteers of 5 to 75 years of age.

The results show that the peak amplitudes of all three b-waves decrease with age, while their implicit times increase with age. These results have been compared to known age-dependent losses in the transmissivity of the crystalline lens of the eye reported by other authors. Changes in lens transmissivity can explain the ERG changes of red/green cone and rod mediated b-waves. In contrast, such lens changes only explain no more than 70% of the changes of the blue cone mediated b-wave.

The relationship between peak amplitude and implicit time changes of the blue cone mediated b-wave indicates that the remaining 30% of the changes (which are not explained by decrease of lens transmissivity with increasing age) may represent a diffuse loss of blue cones in the aging normal human retina.

IV. Blue cone pattern and density and retinal aging in macaques (de Monasterio, Schein, Newlander).

Because of the results reported in the previous section, we started a project to compare blue cone density and pattern distribution in subhuman primates of different ages in order to detect changes that may explain the decrease of blue cone function in the aging human retina.

Preliminary results obtained in rhesus monkeys of an age equivalent to about 75 to 100 years of human age show a clear reduction in the blue cone density and in their packing pattern, using retinas staining with tissue reactive dyes which selectively label blue cones. These reductions, so far, appear to have a differential retinal distribution.

V. Measurements of neutral points and confusion loci in acquired color vision deficiencies (Higgins, Caruso, de Monasterio).

We are developing test systems to allow for the testing of the locale of neutral points (i.e. those wavelengths which are confused with a achromatic light) and the corresponding color confusion loci of acquired cases of color vision deficiencies. Testing of normative values of wavelength saturation discrimination is expected to begin as soon as space for testing becomes available. These values should not only aid in the classification of the acquired deficiency, but should also provide information in the necessary modifications of several routine color vision tests which are based on values

obtained from congenital cases of various deficiencies. Such modifications may be important, as congenital and acquired deficiencies show significant differences in the expression of the underlying alterations.

Significance to Biomedical Research and the Program of the Institute:
The results help the understanding of the mechanisms of acquired color vision defects which preferentially affect blue-sensitive cone function in cases of retinal insult or disease. These results may also assist identification of underlying defect in red and green cone dysfunction as well.

Proposed Course: Studies of color vision defects will be continued.

NEI Research Program: Retinal and Choroidal Diseases--Retinal Organization, Neurotransmission, and Visual Function

Publications:

de Monasterio FM, Schein SJ, McCrane EP: Staining of blue-sensitive cones of monkey retina by a fluorescent dye. Science 213:1278-1281, 1981

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00123-02 CB																																
PERIOD COVERED October 1, 1981 to September 30, 1982																																			
TITLE OF PROJECT (80 characters or less) Psychophysical Studies in Hemianopia																																			
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																																			
<table> <tr> <td>PI: Rafael C. Caruso</td> <td>M.D.</td> <td>Expert</td> <td>CB NEI</td> </tr> <tr> <td>Other: Myles J. Jaffe</td> <td>O.D.</td> <td>Guest Worker</td> <td>CB NEI</td> </tr> <tr> <td></td> <td>Ph.D.</td> <td>Senior Staff Fellow</td> <td>CB NEI</td> </tr> <tr> <td></td> <td>M.D., D. Sc.</td> <td>Chief, Section on Visual Processing</td> <td>CB NEI</td> </tr> <tr> <td>Francisco de Monasterio</td> <td></td> <td></td> <td></td> </tr> <tr> <td>Patricia Mercer</td> <td>B.S.</td> <td>Health Technician</td> <td>CB NEI</td> </tr> <tr> <td>Doris Collie</td> <td>A.A.</td> <td>Health Technician</td> <td>CB NEI</td> </tr> <tr> <td>Mary Fuhrman</td> <td></td> <td>Health Technician</td> <td>CB NEI</td> </tr> </table>				PI: Rafael C. Caruso	M.D.	Expert	CB NEI	Other: Myles J. Jaffe	O.D.	Guest Worker	CB NEI		Ph.D.	Senior Staff Fellow	CB NEI		M.D., D. Sc.	Chief, Section on Visual Processing	CB NEI	Francisco de Monasterio				Patricia Mercer	B.S.	Health Technician	CB NEI	Doris Collie	A.A.	Health Technician	CB NEI	Mary Fuhrman		Health Technician	CB NEI
PI: Rafael C. Caruso	M.D.	Expert	CB NEI																																
Other: Myles J. Jaffe	O.D.	Guest Worker	CB NEI																																
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Doris Collie	A.A.	Health Technician	CB NEI																																
Mary Fuhrman		Health Technician	CB NEI																																
COOPERATING UNITS (if any) Department of Radiology, CC Branch of Developmental Endocrinology, NICHD																																			
LAB/BRANCH Clinical Branch																																			
SECTION Section on Visual Processing																																			
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205																																			
TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0.0																																	
CHECK APPROPRIATE BOX(ES)																																			
<input checked="" type="checkbox"/> (a) HUMAN SUBJECTS		<input type="checkbox"/> (b) HUMAN TISSUES	<input type="checkbox"/> (c) NEITHER																																
<input type="checkbox"/> (a1) MINORS		<input type="checkbox"/> (a2) INTERVIEWS																																	
SUMMARY OF WORK (200 words or less - underline keywords)																																			
<p>The visual function of patients with <u>chiasmatic</u> and <u>retrochiasmatic</u> lesions of the visual pathways is assessed with <u>psychophysical</u> tests. These include <u>kinetic</u> and <u>static perimetry</u>, <u>color vision</u> tests and <u>spatial contrast sensitivity</u> studies. The purpose of this study is to identify and develop tests to characterize the nature and evolution of <u>visual loss</u> in lesions that cause hemianopia.</p>																																			

Project Description:

Objectives: The aim of this project is to identify the patterns of visual loss and patterns of visual recovery in patients with chiasmal and retro-chiasmal lesions of the visual system which determine hemianopic field defects, and to develop the psychophysical tests best suited to monitor these functional changes.

Methods Employed: 1. Perimetry: The visual fields are explored with kinetic quantitative perimetry and static quantitative perimetry. In this latter procedure, the differential threshold for light perception is determined in a set of points in the visual fields to either side of the central vertical meridian, using a staircase method to identify the threshold. 2. Color vision: Central vision is estimated using the following methods: AO-HRR pseudoisochromatic plates, Farnsworth-Munsell D-15 panel, Farnsworth-Munsell 100-Hue test and the Nagel anomaloscope. 3. Spatial contrast sensitivity: The spatial contrast sensitivity function is determined using sinusoidal luminance gratings with spatial frequencies between 0.9 and 24 cycles per degree generated on an oscilloscope screen. A two-alternative temporal forced-choice technique is used for a criterion-free judgment of threshold visibility.

The psychophysical findings are correlated with the neuro-radiological estimation of the topography and size of the lesion.

Major Findings: Patients with central hemifield losses identified with either kinetic or static perimetry show alterations of their contrast sensitivity function and their color vision. These alterations are infrequently seen in the absence of a field loss. Perimetry, then, seems to be a sensitive indicator of the presence of a functional loss although it does not provide a complete characterization of the deficit.

Improvements of the psychophysical parameters tested are seen after successful treatment with radiologically documented reduction of the volume of the lesion. A reduction of the contrast sensitivity threshold and an improvement of color discrimination are associated with the perimetric recovery.

Significance to Biomedical Research and the Program of the Institute: Psychophysical tests provide an accurate and non-invasive procedure to monitor the evolution of regression of intracranial lesions that involve the visual pathways. Frequently, as in the case of sellar lesions, the mode of therapy selected depends on the presence of visual symptoms. Therefore, the characterization of the patterns of early visual loss and the identification of the most sensitive indicators of this deficit are expected to be of value in the management of these disorders. These results may also provide information about the physiopathology of the visual deficit in hemianopia.

Proposed Course: Psychophysical studies of visual function in chiasmal and retrochiasmal lesions will be continued, introducing modifications of the techniques described that are expected to improve their diagnostic value.

Project No. Z01 EY 00123-02 CB

NEI Research Program: Strabismus, Amblyopia and Visual Processing--
Visual Processing and Amblyopia (Disorders)

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00144-01 CB

PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Visual Evoked Responses in Lesions of the Visual Pathways

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Rafael C. Caruso	M.D.	Expert	CB	NEI
Other:	Lezheng Wu	M.D.	Visiting Fellow	CB	NEI
	Francisco M. de Monasterio	M.D., D.Sc.	Chief, Section on Visual Processing	CB	NEI
	Myles J. Jaffe	O.D.	Guest Worker	CB	NEI
	Patricia Mercer	M.S.	Health Technician	CB	NEI
	Doris Collie	A.A.	Health Technician	CB	NEI
	Mary Fuhrman		Health Technician	CB	NEI

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Branch

SECTION

Section on Visual Processing

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0.0

CHECK APPROPRIATE BOX(ES)

 (a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER (a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Visual evoked responses are recorded in normal volunteers and in patients with lesions of the retina, optic nerves, optic chiasm, optic radiations and visual cortex. Both pattern stimuli and unstructured stimuli are used. The recordings are used for diagnostic purposes and to provide an objective assessment of visual function in these conditions. These data are correlated with the results of psychophysical tests of visual function.

Project Description:

Protocol Number: 82 EI 55

Objectives: The aim of this project is to characterize the normal visual evoked response elicited by different stimuli and to analyze the patterns of its alteration in lesions of the visual pathways.

Methods Employed: Visual evoked responses are elicited by pattern reversal stimuli (checkerboards) displayed in a television screen or sine-wave gratings generated on an oscilloscope screen and by unstructured stimuli (achromatic and chromatic flashes). Recordings of the electrical activity of the occipital lobes made after each stimulus presentation are digitized and averaged to isolate the components of the visual evoked response.

Major Findings: Data from normal volunteers of different age groups are currently being collected and analyzed. The degree of symmetry of the response elicited by stimulation of each eye and that of the response recorded from each cerebral hemisphere are being quantified, as well as the test-retest reproducibility of the results.

Visual evoked responses recorded from patients with lesions of the visual pathways are being correlated with the results of psychophysical evaluation of visual loss. These subjects include a population of patients with oculo-cutaneous albinism and a population of patients with ocular hypertension and open angle glaucoma.

Significance to Biomedical Research and the Program of the Institute: Visual evoked responses provide a sensitive and non-invasive procedure for the diagnosis and follow-up of ophthalmological and neuro-ophthalmological syndromes. The identification of the most suitable stimulation and recording parameters and the analysis of the patterns of alteration of the response in lesions of the visual pathways are expected to enhance the diagnostic value of this technique.

Proposed Course: Recordings of visual evoked responses in patients and normal subjects will be continued, introducing modifications of the techniques described that are expected to improve their diagnostic value.

NEI Research Program: Strabismus, Amblyopia and Visual Processing--Visual Processing and Amblyopia Disorders

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00006-11 CB
PERIOD COVERED October 1, 1981, to September 30, 1982		
TITLE OF PROJECT (80 characters or less) Research in Methods of Evaluating Visual Processes		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Ralph D. Gunkel Other: David G. Cogan Fred C. Chu David A. Newsome	O.D. Ophthalmic Physicist M.D. Medical Officer M.D. Senior Staff Fellow M.D. Senior Staff Fellow	CB NEI CB NEI CB NEI CB NEI
COOPERATING UNITS (if any)		
LAB/BRANCH Clinical Branch		
SECTION Section on Visual Processing		
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 1.5	PROFESSIONAL: 1.5	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)		
<p>Appropriate <u>psychophysical procedures</u> are used to measure the various visual functions of patients in the Eye Clinic, particularly <u>thresholds of visibility</u> for the <u>retinal rods and cones</u> and for <u>discrimination of colors</u>, all under standard conditions. These tests are done for the purpose of discovering and monitoring any changes in visual efficiency due to <u>degenerative diseases</u> or <u>toxic medications</u>. Efforts continue in attempts to find or devise test procedures which are more effective, more objective, and less demanding on the patients.</p> <p>Tests were conducted on 377 patients during the past year.</p>		

Project Description:

Protocol Number: 80 EI 08

Objectives: The major objective is to determine by, the most sensitive and reliable psychophysical methods available, the precise visual condition of eye patients as a guide to their medical management.

A secondary objective is the relative evaluation of the chromagraph and other devices in terms of sensitivity, consistency, and convenience.

Methods Employed: All patients referred for psychophysical testing are given the Hardy-Rand Rittler Pseudoisochromatic test for color blindness, the Farnsworth Dichotomous Test Panel D-15, the Nagel anomaloscope for the Rayleigh equation, and the Gunkel Chromagraph test for color discrimination. In cases where it is indicated, dark adaptation and cone thresholds are measured with the modified Goldmann-Weekers adaptometer.

In Dr. Newsome's macular degeneration project the data became so massive as to require processing for computer-handling.

Major Findings: Measurement of cone thresholds appears to be our most sensitive index for monitoring the macular condition for the following cases of toxic or degenerative retinopathies. Final thresholds in dark adaptation give the best measurement of rod sensitivity in night-blinding diseases.

The Hardy-Rand Rittler Pseudoisochromatic plates and the Farnsworth Panel D-15 are fairly good for screening color defects, but they are not good for quantitating and they sometimes miss subtle defects which are found with the Gunkel chromagraph. The Nagel anomaloscope purports to measure only red and green defects, so is useless for the most common color defect found in the eye clinic, namely yellow. A condition known as "pseudo-protanomaly" has become common in the literature relating to the anomaloscope, and that along with the fact that subjects with severe anomalies see no color whatever in the small test spot tend to make the instrument less useful in the eye clinic.

The chromagraph plots discrimination thresholds for as many colors as desired in a matter of minutes, is very sensitive, and is remarkably consistent in repeated tests.

Significance to Biomedical Research and the Program of the Institute:
A poster on "Quantitating Defects in Color Vision" was shown at the ARVO meeting in May.

The conventional color-vision tests have been useful primarily for screening to determine the presence or absence of significant genetic defects, and hopefully to differentiate protanopia, deutanopia, and tritanopia. These tests have not been successful in describing the less severe defects, and separating protanopes from deutanopes is of doubtful value, since current investigators state that there is no significant difference in their perception of red and green as colors. Since this separation is made largely on the basis

of apparent luminosity, it gives little information regarding the colors the subject actually perceives, which is what we really wanted to learn in the first place.

The chromagraph is the first clinical instrument to reveal the exact saturation required for the discrimination of each color (not just red and green), since it is based entirely on colors perception. It is believed that a new trend will now develop to replace the grossly ambiguous terms of "protanopia" and "deutanopia" with specific color names at designated saturation levels in describing defects in color vision. Since subtle defects not revealed by the other tests can now be quantitated, correlations are being found between specific color defects and certain ocular and systemic disorders.

A paper entitled "Clinical Studies of Color Vision Using the Gunkel Chromagraph" by Fred C. Chu, M.D., David G. Cogan, M.D., and Douglas Reingold, M.A., has been accepted for publication by the Archives of Ophthalmology.

An extensive study relating melatonin production to light exposure is in progress in another institute, and our psychophysical tests are utilized in repeated tests on the subjects.

Proposed Course: It appears desirable to continue the project more or less in its present form. The clinical studies are needed, and the data being acquired add to the evidence correlating subtle color defects with specific disease entities.

For those who question the validity of the chromagraph results, the only answer lies in further testing.

NEI Research Program: Retinal and Choroidal Diseases--Retinal Organization, Neurotransmission, and Visual Function

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00121-02 CB																																				
PERIOD COVERED <u>October 1, 1981, to September 30, 1982</u>																																							
TITLE OF PROJECT (80 characters or less) <u>Spatial Contrast Sensitivity Studies in Retinal and Neuro-ophthalmological Disease</u>																																							
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																																							
<table> <tr> <td>PI:</td> <td>Kent E. Higgins</td> <td>Ph.D.</td> <td>Senior Staff Fellow</td> <td>CB</td> <td>NEI</td> </tr> <tr> <td>Other:</td> <td>Francisco M. de Monasterio</td> <td>M.D., D.Sc.</td> <td>Chief, Section on Visual Processing</td> <td>CB</td> <td>NEI</td> </tr> <tr> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> </tr> <tr> <td></td> <td>Myles J. Jaffe</td> <td>O.D.</td> <td>Guest Worker</td> <td>CB</td> <td>NEI</td> </tr> <tr> <td></td> <td>Rafael C. Caruso</td> <td>M.D.</td> <td>Expert</td> <td>CB</td> <td>NEI</td> </tr> <tr> <td></td> <td>Brooke Shefrin</td> <td>B.S.</td> <td>Guest Worker</td> <td>CB</td> <td>NEI</td> </tr> </table>				PI:	Kent E. Higgins	Ph.D.	Senior Staff Fellow	CB	NEI	Other:	Francisco M. de Monasterio	M.D., D.Sc.	Chief, Section on Visual Processing	CB	NEI								Myles J. Jaffe	O.D.	Guest Worker	CB	NEI		Rafael C. Caruso	M.D.	Expert	CB	NEI		Brooke Shefrin	B.S.	Guest Worker	CB	NEI
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SECTION <u>Section on Visual Processing</u>																																							
INSTITUTE AND LOCATION <u>National Eye Institute, NIH, Bethesda, Maryland 20205</u>																																							
TOTAL MANYEARS: 2.3	PROFESSIONAL: 2.3	OTHER: 0.0																																					
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<input type="checkbox"/> (a1) MINORS		<input type="checkbox"/> (a2) INTERVIEWS																																					
SUMMARY OF WORK (200 words or less - underline keywords)																																							
<p>The purpose of this project is to provide <u>diagnosis</u> assessment of deficits and alterations in <u>visual resolution</u> in <u>toxic</u>, <u>inflammatory</u>, <u>degenerative</u>, or <u>congenital</u> <u>retinal</u> and <u>neuro-ophthalmological</u> disorders through the measurement of overall <u>spatial contrast sensitivity</u>.</p>																																							

Project Description:

Objectives: Detection of deficits and changes in spatial contrast sensitivity in toxic, inflammatory, degenerative, and congenital visual disorders affecting the retina and higher visual pathways. Development of age and sex norms for normal subjects. Evaluation of different psychophysical methods with respect to test-retest reliability. Evaluation of effects of artificial scotomata on contrast sensitivity in normal subjects under conditions of stabilized retinal imagery.

Methods Employed: Two different psychophysical techniques are being used to measure spatial contrast sensitivity in normal volunteers and clinical patients. The first technique (method-of-adjustment) is in common use since it does not require a computer and, presumably, takes less time. Unfortunately, it is not possible to evaluate the exact frequency-of-seeing criterion (or changes therein) that a given patient may be using as a basis for the judgment of threshold visibility. Consequently, changes in sensitivity across spatial frequencies and across time may reflect criterion fluctuations and not a change in the status of the visual pathways. The second technique (forced-choice), in contrast, is essentially free from patient criterion fluctuations. This technique is less commonly used in a clinical setting, primarily because it is extremely time-consuming unless stimulus presentation and data storage are under computer control as it is in this study.

Similar procedures are being used to study the effect of artificial scotomata on spatial contrast sensitivity in normal subjects. For these measurements, a Double-Purkinje-image Eyetracker System is used to stabilize the retinal image of the CRT stimulus display. Spatial contrast sensitivity is measured without and then with portions of the grating display masked to simulate scotomata of varying sizes, shapes, and locations.

Major Findings: For normal subjects, our results indicate that an automated forced-choice technique does not require any more patient test time than is required by the more conventional method-of-adjustment. In addition, the forced-choice procedure evidences superior test-retest repeatability. Using the method-of-adjustment, approximately 20-30 percent of normal volunteers have evidenced significant test-retest shifts in contrast sensitivity. These shifts are of sufficient magnitude that, if observed in patients, would lead to either false positive or false negative diagnoses. Similar shifts are not observed when the same subjects are tested with the forced-choice technique.

During this past year, the forced-choice method has been used almost exclusively for patient testing. These results indicate that the method can be used with equal efficiency and reliability for testing naive patients with disorders of diverse etiology (e.g., macular degeneration, uveitis, and gyrate atrophy).

Results of testing normal subjects under artificial scotoma conditions are being collected to provide a baseline for predicting the particular type and magnitude of contrast sensitivity loss to be expected in patients having diverse types of visual field loss. Preliminary results indicate, for example,

that the type of contrast sensitivity loss obtained with an artificial central scotoma (predominantly high frequency or overall) depends critically on temporal factors associated with grating presentation. A predominantly high frequency loss was obtained when grating contrast was turned on and off gradually. An additional low frequency difference was obtained when grating contrast was turned on and off abruptly.

Significance to Biomedical Research and the Program of the Institute: This project will facilitate diagnosis and follow-up evaluation of retinal and neuro-ophthalmological disorders in inpatients, outpatients and referred patients of the NIH Eye Clinic. Of particular significance are findings that the constant-criterion forced-choice technique is likely to represent the most sensitive and reliable method for the early-detection and follow-up of contrast sensitivity changes produced by diverse ocular disorders and that the type of spatial contrast sensitivity loss produced by an artificial scotoma depends importantly on temporal factors.

Proposed Course: Studies of contrast sensitivity will be continued, with particular emphasis given to testing of development of large-field normative data with particular emphasis being given to the determination of the pattern and magnitude of sensitivity loss characteristic of various visual disorders and to the development of additional procedures for measurement of residual vision in patients having severe sensitivity losses.

NEI Research Program: Strabismus, Amblyopia, and Visual Processing-Visual Processing and Amblyopia(Disorders)

Publications:

Higgins KE, Daugman JG, Mansfield, RJW: Amblyopic contrast sensitivity: Insensitivity to unsteady fixation. Invest Ophthalmol Vis Sci 23: 113-120, 1982.

Higgins KE, Caruso RC, Coletta NJ, de Monasterio FM: Effect of an artificial central scotoma on spatial contrast sensitivity in normal subjects. Invest Ophthalmol Vis Sci 22 (Suppl):252, 1982.

Coletta NJ, Higgins KE, Jaffe MJ, Caruso RC, de Monasterio FM: Criterion-measurement of spatial contrast sensitivity in a clinical population. Invest Ophthalmol Vis Sci 22 (Suppl):217, 1982.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

J.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00122-02 CB

PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Anatomical Studies of the Visual System of Primates

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Stanley J. Schein	M.D., Ph.D.	Expert	CB	NEI
Other: Francisco de Monasterio	M.D., D.Sc.	Chief, Section on Visual Processing	CB	NEI
Rafael C. Caruso	M.D.	Expert	CB	NEI
Edna P. McCrane	B.S.	Biologist	CB	NEI
Marvin B. Shapiro	M.A.	Research Mathematician	LSMM	DCRT
Jim E. Fullbrook	Ph.D.	Guest Worker	CB	NEI
J. Kelly Newlander		Summer Student	CB	NEI
Jorge A. Martinez	B.S.	Technician	CB	NEI

COOPERATING UNITS (if any)

Laboratory of Statistical and Mathematical Methodology, DCRT

LAB/BRANCH

Clinical Branch

SECTION

Section on Visual Processing

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS

(b) HUMAN TISSUES

(c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The aim of this project is to study the functional anatomical organization of neurons of the visual system of non-human primates that can serve as a model for the human visual system. Parcellation of the monkey visual cortex is based on silver cell, silver myelin and cytochrome oxidase staining and on-connectional studies. Cytochrome oxidase staining is also being used to activity label chronic stimulation states in the brain, and improvements in the 2-deoxy-glucose activity labelling method for acute stimulation states has been developed. Further improvements are in progress. Retinal studies have focused on blue-sensitive cones, their pattern, synaptology, and the mechanism by which blue-sensitive cones may be specifically stained.

Project Description:

Objectives: To study the neural organization underlying visual processing in retina and cortex.

Methods Employed: Silver cell, silver-myelin and cytochrome oxidase staining of monkey visual cortex; activity labelling of chronic states with cytochrome oxidase; activity labelling of acute states with 2-deoxyglucose; intravitreal injection of dyes; histological processing of retina, including whole mounts, plastic and celloidin; computer modelling and statistical analyses of patterns; electron microscopy; image processing technology.

Major Findings:

I. Parcellation of visual cortex:

Cell and myelin stains have been used traditionally to subdivide the cerebral cortex into areas. This approach assumes that the pattern of elements reflects differences in function and connectivity among individual visual areas. Dissatisfaction with the so-called architectonic approach was derived from two sources. First, the staining patterns were not sharp, and borders (subtle in fact) were difficult to mark with certainty. Second, confirmatory evidence for the assignment of borders on the basis of functional and connectional differences was unavailable.

A. Silver staining for parcellation of visual cortex (Schein, de Monasterio, McCrane, Martinez).

Recent discoveries of silver methods for cell staining (Merker's method) and myelin staining (Gallyas' method) provide powerful tools for study of the cyto-and myelo-architecture of visual cortex. Further, we have markedly improved the new silver cell stain to the point where the neuronal soma are stained in their entirety, along with the initial portions of primary processes. Striate visual cortex was used as a testing ground for the improved silver cell stain: We identified and photographed representative examples of every cell type in striate cortex. We have begun applying these two powerful staining methods to extrastriate visual cortex in both old world and new world monkeys.

B. Activity-labelling for parcellation of visual cortex (Schein, de Monasterio, McCrane).

Cytochrome oxidase, a mitochondrial enzyme, provides staining patterns based on mitochondrial labelling in cells of cerebral cortex. The intensity of staining reflects chronic (aerobic) activity. Areas of visual cortex differing in function and positioning of mitochondrial reservoirs (cell bodies and nerve terminals) would be expected to differ in their cytochrome oxidase staining patterns. The patterns are not crisp, since the "background" of mitochondria is high, but borders of extrastriate visual areas can be marked. Borders based on cytochrome oxidase staining reflect a combination of structural and long-term functional differences between visual areas. The 2-deoxyglucose method, described below, should mark borders based on short-term functional differences.

II. Activity-labelling of central nervous system

A. Acute studies with 2-deoxyglucose (Schein, de Monasterio).

We have been developing and studying the principles of techniques aimed at improving the resolution of the quantitative deoxyglucose method. The method, developed by Sokoloff, Kennedy and their associates at the NIH, results in autoradiographic mapping of glucose utilization. Such a "glucogram" reflects metabolism modulated by short-term nervous activity. The coarse autoradiographic resolution presently available smears local peaks and valleys of activity. We expect that with fine resolution glucograms (giving higher peaks and lower valleys as well as crisper patterns) it will be possible to assign functional roles to the different areas of the visual cortex and to begin studying retinal circuitry and patterns.

Each of the steps proposed in the development of fine-resolution glucography must operate within severe constraints: The large size of the tissue involved, the necessity of quantitative retention and of strict localization of the water-soluble label. We used the ocular dominance system of the striate cortex of marmoset monkey as a model system for verification of the new methodology. We obtained glucograms of this (histologically uniform) tissue, showing dramatic variations in glucose utilization, corresponding to the ocular dominance columns. In addition, cortical layers and some sub-layers were relatively clearly demarcated.

In order to achieve these results, we solved the following problems: fixation of tissue to prevent cells from becoming leaky after sacrifice of the animal; the physical mechanism of replacement of tissue-water by solvent at cold temperature; the organic chemistry of withdrawal of labelled 2-deoxy-D-glucose-6-phosphate by solvents; embedment of tissue, sectioning and mounting of sections according to these chemical rules; calibration of autoradiographic density to obtain tissue tracer concentration by a method which does not use 3-O-methyl-glucose.

B. Chronic studies with cytochrome oxidase (McCrane, Schein, de Monasterio).

The richness of mitochondrial enzymes, among them cytochrome oxidase, in brain is a function of chronic aerobic demand. We enucleated two monkeys and waited two months before sacrifice. Portions of the striate visual cortices of these monkeys and of two normal monkeys were sectioned perpendicular and parallel to the surface. The normal pattern of staining was radically altered in the unilaterally enucleated monkeys. Among the many changes, most noteworthy was the staining of layer 4CB, the layer of striate cortex which receives a heavy parvocellular lateral geniculate projection. When sectioned parallel to the surface, through layer 4CB, the slabs of ocular dominance columns were

readily visualized. This example indicated that obvious changes could be observed after long-term changes of visual function. We also studied correlations between the changes observed using cytochrome oxidase reaction in unilaterally enucleated monkeys and the 2-deoxyglucose activity in monkeys with one eye occluded.

III. Studies of blue-sensitive cones.

A. Mechanism of blue-cone selectivity of procion staining (Schein, de Monasterio, Caruso, McCrane).

Köllner's Rule notes that disease of the retina causes a "blue-yellow" defect, which suggested that blue-sensitive cones are particularly vulnerable to retinal insult. We hypothesized that the blue cones were most sensitive to the chemical toxicity of tissue-reactive dyes like Procion yellow (PY). When the blue cones died, the stain penetrated to give a complete staining, producing Golgi-like silhouettes of the blue cones. To test the hypothesis that Procion yellow stained cells were leaky we used a dye that can be used as a "leakiness detector", i.e. dye which should not stain photoreceptors in a properly handled, normal retina but should stain leaky cells. In unstained eyes, no leaky cells were detected in central retina. In Procion yellow stained eyes, only the PY-stained cones (the blue cones) were concurrently stained by the leakiness-detecting dye. Thus, (blue) cones stained with Procion yellow were leaky, while the other (red and green) cones were not.

The concentration dependence of the blue-cone selective staining pattern was studied. The "window" of effective dye concentration was over a 2.5 fold range. Below that window, only cone outer segments stained; above the window, rods and eventually other cones were stained. The concentration-dependent pattern was similar for several other dyes which could be used to selectively stain the blue cones. We regard these findings as further evidence for a "toxicity" mechanism underlying the intracellular staining.

In portions of mistreated retina, pre-injected only with the above-described leakiness detecting dye, areas of apparently selective staining of blue cones were observed. We regard this finding as evidence that the blue cones are indeed more vulnerable than the rods and other cones, consistent with the concentration thresholds for staining each of these elements. We also noted that Procion yellow failed to stain cells which were made leaky by death following enucleation. We hypothesized that no free dye remained at 24 hours after intravitreal injection. We confirmed this hypothesis by removing and mis-handling eyes after shorter incubations and finding dyestaining of all cones as well as blue cones, in fact, in all retinal cells.

We are using these insights to assist development of a macaque monkey free of blue cones. Such animals would be of use in basic psychophysical and physiological studies as well as in the development and understanding of clinically-related electro-diagnostic methods.

B. Analysis of blue-sensitive cone pattern (Schein, Shapiro, de Monasterio).

A pattern of points, such as that described by the blue cones, may be described as irregularly or regularly spaced. Furthermore, the pattern may be described according to a model which generates a similar pattern. The simplest models of a regular pattern are triangular, square and hexagonal packing. More complex models discuss the packing of "hard spheres," "soft spheres" etc. How best to describe a spatial pattern is a problem of widespread theoretical interest, particularly to biologists. We whole-mounted blue-cone-stained monkey retinas, without distortion or loss of labelled cones. We were able to quantify the disorder present in the blue cone pattern, and to show that no simple lattice models with the appropriate normal-disorder matched the blue cone pattern in its lattice structure; a "ball" model was generated which matched the blue cone pattern in both its normal-disorder and its lattice structure; the "ball" has a hard core of diameter roughly equal to half of the average distance between neighboring blue cones. (It should be noted that the physical diameter of the blue cone is about 20% of the average separation between blue cones.) The hard-core was surrounded by a soft shell which extended out to this average distance of separation. The results show placement of the center of a blue cones is (i) prohibited within the hard core of another blue cone, (ii) possible according to a probability relation within the soft shell and (iii) is completely acceptable outside the soft shell. This model, which has only a single parameter, is an excellent model for the blue cone pattern and has interesting biological features. In particular, the soft shell can be described in terms of repulsion which obeys an inverse-square relation.

Comparison of models to the blue cone pattern was possible by a procedure involving 5 steps. 1. Digitization of the locations of the blue cones. 2. Generation of informative model patterns, like normally-disordered square-packed points etc. 3. Application of a disorder sensitive statistic to the patterns. 4. Application of a lattice-structure sensitive statistic--our invention--to the pattern. 5. Display of all statistics as cumulative density functions. This method of analysis is extremely powerful and should prove generally useful in pattern analysis, both within biology and without.

C. Blue-cone staining and post-receptoral staining by different tissue-reactive dyes in monkey retina (de Monasterio, Schein, Caruso, Newlander).

We have found that dyes other than Procion yellow are able to stain blue cones. These various dyes differ in the pattern of post-receptoral staining, mostly of bipolar and horizontal cells. Emphasis has been given to Procion black, a non fluorescent, electron-dense dye that can be observed both with light- and electronmicroscopy. Differently from Procion yellow, Procion black produces what appears to be a rather selective staining of a population of horizontal cells which are always located in the close vicinity of a stained blue cone. A similar result has been obtained with a fluorescent dye, Lucifer yellow VS. Combination of the results obtained so far with these dyes indicate that any given blue cone may be in contact with a single horizontal

cell which is also in synaptic contact with red and/or green cones (not stained by the dyes). On the average, each of these horizontal cells contact between 6 to 9 cones, one of them being a blue cone. These results are relevant to recent findings indicating that blue cone signals only have access to color-opponent postreceptoral pathways as early as the outer plexiform layer.

Other dyes under study produce somewhat different postreceptoral staining patterns. Preliminary results indicate that these differences may be related to different pathways use by the dyes to reach the outer-most layers of the retina.

D. Electronmicroscopic studies of monkey blue cones (Caruso, de Monasterio, Fullbrook, Schein).

We have found two electron-dense dyes which stain blue cones as does Procion yellow. Electron microscopic studies of the ultrastructure of stained retinas are in process now. The blue-cone system differs from the red and green-cone system in a number of psychophysical and physiological properties.

Investigation of the synaptology of the blue cones may provide insight into some of these properties.

Significance to Biomedical Research and the Program of the Institute:

Understanding the organization of the visual system of non-human primates is valuable for the understanding of the human visual system. Functional description of cortical organization in monkey should, in itself, provide insight in human visual deficiencies. More specifically, a positron-emitter labelled 2-deoxyglucose is already in use for non-invasive activity labelling in humans. The resolution of the PET scans is poor at present, nearly one centimeter, and the theoretical limit is about five millimeters. Understanding the function of separate visual areas in monkey is therefore of special importance to maximally and differentially stimulate entire areas, encompassing large volumes of tissue.

We have found two electron-dense dyes which stain blue cones as does Procion yellow. Electron microscopic studies of the ultrastructure of stained retinas are in process now. The blue-cone system differs from the red and green-cone system in a number of psychophysical and physiological properties. Investigation of the synaptology of the blue cones may provide insight into some of these properties.

Proposed Course: All of the neuroanatomical studies described will be continued. Retinal studies, already productive, should continue to be so. The cortical studies are just now at a stage where their promise may be fulfilled.

Laboratory of Molecular and Developmental Biology

ANNUAL REPORT
NATIONAL EYE INSTITUTE
October 1, 1981 - September 30, 1982

REPORT OF THE CHIEF, LABORATORY OF MOLECULAR AND DEVELOPMENTAL BIOLOGY
Joram Piatigorsky, Ph.D.

FY 1982 marks the first year for the new Laboratory of Molecular and Developmental Biology (LMDB). The members of the LMDB have formed a cohesive group and occupy quarters adjacent to the Laboratory of Vision Research (LVR) in Building 6. Although newly born, the LMDB has been very active and has begun to interact with the LVR. For example, a joint LMDB-LVR weekly seminar series was given throughout the year in which the members of both laboratories became acquainted with each other's work and shared ideas. In addition, LVR members have worked closely with the LMDB in attempting to clone a rhodopsin cDNA (Mr. J. Alligood) and in investigating a defect in β -crystallin gene expression in the Philly mouse cataract (Dr. J.H. Kinoshita and Ms. D. Carper).

The LMDB is not divided formally into sections, but has four independent, interacting groups. Together, they explore the molecular genetics and biochemistry of the visual system. Particular attention is given to eye development and, when possible, disease. For instance, the regulation of crystallin synthesis is studied in both normal, embryonic lenses and hereditary cataractous lenses. The structure, organization, expression, and evolution of crystallin genes is also under investigation.

In addition to the crystallin genes, collagen and rhodopsin genes are presently being explored by the LMDB. Moreover, phospholipid metabolism within the cell membrane is under investigation with respect to lens cell differentiation. These studies may ultimately link metabolic events at the cell surface with differential gene expression, providing a comprehensive view for the control of differentiation of lens cells. The LMDB thus examines the visual system in terms of its genes and uses the eye as a model for understanding the molecular and developmental genetics of eukaryotic cells.

The major accomplishments of the four groups in the first year of this laboratory are as follows. My group has cloned cDNAs for representatives of all the crystallin families and has cloned genes or segments of genes for α -, β - and δ -crystallin polypeptides. Sequencing studies have provided the first primary structures for mouse α - and β -crystallin polypeptides and chicken δ -crystallin polypeptides, and have strongly supported the novel idea that the β - and γ -crystallins form a superfamily of related proteins sharing a common ancestral gene that underwent an intragenic duplication early in its evolutionary history. The first view of a mammalian (mouse) β -crystallin gene revealed three intervening sequences. The resulting four exons each code for one of the predicted folding units of the protein, linking gene structure with protein structure. Collaboration with Dr. T. Blundell (Birkbeck College, University of London) led to a computer-derived model of the tertiary structure of this β -crystallin polypeptide, indicating strong homology to the γ -crystallins and a remarkably hydrophobic N-terminal extension. The latter suggests that this β -crystallin polypeptide may interact with the cell membrane. Studies showed differential gene regulation within the β -crystallin gene family during chicken and mouse lens development, and demonstrated selective loss (gene inactivation?)

of δ -crystallin mRNA in chicken lenses three to five months after hatching. A specific defect in β -crystallin gene expression, noted above, was found as the earliest lesion reported yet in the development of the Philly mouse hereditary cataract. Finally, evidence has been obtained for a new crystallin in the turtle lens.

The group headed by Dr. Peggy Zelenka has discovered two changes in phospholipid metabolism that accompany lens fiber cell differentiation: a transient burst in the transmethylation of phosphatidylethanolamine and a stabilization of phosphatidylinositol. A transient increase in phosphatidylethanolamine transmethylation occurs within 6 seconds after the induction of lens fiber cell differentiation *in vitro*, and may initiate the process. Further experiments suggest that lipoxygenase metabolites of arachidonic acid may be involved in regulating lens fiber cell differentiation. These experiments provide the first handle on the events controlling lens fiber cell development and implicate the cell surface as a regulatory site for differentiation.

A happy event for the LMDB was the conversion of Dr. Toshimichi Shinohara to a tenured position. Dr. Shinohara and his colleagues have established cDNA libraries of rat and bovine retina. These are being screened for opsin cDNAs, which will provide the key to the genome for these important photoreceptive proteins. In addition, this group has shown recently that retinal binds to bovine γ -crystallin with an apparent Schiff base linkage. This surprising result has implications for understanding the nature of retinal binding to opsin and, possibly, cataractogenesis.

Finally, Dr. Gabriel Vogeli has established a library of high molecular weight cDNAs from the chicken embryo. Screening tests with large RNAs (>5000 nucleotides) from the lens and from chorioallantois membranes have resulted in the isolation of several cloned cDNAs that may encode type IV (basement membrane) collagen. Identification of basement membrane collagen cDNA will allow determination of the primary structure of type IV collagen, isolation of its gene(s), and studies concerning the regulation of the synthesis of basement membrane within eye tissues.

Thus, the LMDB has a good start and is happy to be part of the NEI. We anticipate that mutual interests, proximity, and complementary skills will lead to an exciting symbiosis between the LMDB and other NEI laboratories in the years ahead.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00126-01 LMDB

PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Crystallin Genes: Structure, Organization, Expression, and Evolution

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Joram Piatigorsky	Ph.D.	Chief	LMDB	NEI
Others:	J. Fielding Hejtmancik	M.D.	Medical Staff Fellow	LMDB	NEI
	Jacques Treton	Ph.D.	Fogarty Fellow	LMDB	NEI
	George Inana	M.D.	Research Associate	LMDB	NEI
	John M. Nickerson	Ph.D.	Staff Fellow	LMDB	NEI
	Raymond E. Jones	Ph.D.	Staff Fellow	LMDB	NEI
	Charles R. King	B.S.	Chemist	LMDB	NEI
	Leah A. Williams	Ph.D.	Guest Worker	LMDB	NEI
	Barbara Norman	B.S.	Chemist	LMDB	NEI
	Toshimichi Shinohara	Ph.D.	Biologist	LMDB	NEI
	Jin H. Kinoshita	Ph.D.	Scientific Director		NEI
	Deborah A. Carper	B.A.	Biologist	LVR	NEI

COOPERATING UNITS (if any)

Joseph Horwitz, Jules Stein Eye Institute, UCLA Medical School; Jacob V. Maizel, Jr., LMG, NICHD; Tom Blundel, Birkbeck College, University of London.

LAB/BRANCH

Laboratory of Molecular and Developmental Biology

SECTION

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: PROFESSIONAL: OTHER:

8.0 6.1 1.9

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The structure, organization, expression, and evolution of the genes for the crystallins of the eye lens have been examined. Sequencing of cDNAs for mouse α A- and β -crystallin and chicken δ -crystallin polypeptides have provided primary structures for these lens proteins. The exon-intron structure of a mouse β -crystallin gene was shown to relate to the folding units predicted for its protein and differed greatly from that of the highly interrupted δ -crystallin genes in chickens and ducks. Analysis of cDNAs encoding four mouse γ -crystallin and four chicken β -crystallin polypeptides revealed that these proteins have related gene families which probably arose from a common ancestral β / γ -gene that internally duplicated. A specific deficiency of a 27K β -crystallin mRNA was found in the Philly mouse cataract, and a selective loss of δ -crystallin mRNA was shown in chicken lenses three to five months after hatching. Evidence has been obtained for a new crystallin in the turtle lens. Together, the data extend our knowledge of the molecular genetics of the crystallins.

Project Description:

Objectives: The objectives of this project are to understand the structure, organization, expression, and evolution of the gene families encoding the lens crystallins. Particular attention is given to the regulation of crystallin gene expression during lens development and, when possible, to defects in gene function during cataractogenesis.

Methods Employed: Conventional methods for analysis of proteins and nucleic acids are used. These include polyacrylamide gel electrophoresis, isoelectric focussing, protein fingerprinting, RNA and DNA isolation, molecular hybridization, cell-free synthesis, molecular cloning, DNA sequencing, and electron microscopy. Chickens, mice, and turtles are used as experimental animals.

Major Findings:

1. A cDNA for the αA_2 -crystallin polypeptide of the mouse has been sequenced. This revealed that the 14s mRNA is monocistronic and contains twice as many non-coding as coding sequences. The noncoding sequences are 3' to the coding sequences.

2. A mouse αA -crystallin gene has been cloned and partially sequenced.

3. A cDNA for a major mouse β -crystallin polypeptide has been sequenced. The primary structure of this polypeptide has been shown to contain an internal duplication, as occurs in bovine γ_{II} -crystallin. Considerable homology was noted with the bovine βBp - and γ_{II} -crystallin polypeptides. Thus the β - and γ -crystallins form a superfamily of lens proteins which apparently arose from a common precursor gene that internally duplicated.

4. The gene for the mouse β -crystallin polypeptide analyzed above has been cloned and partially sequenced. It contains four exons. The tertiary structure of the protein contains four symmetrical folding units (like bovine γ_{II} -crystallin), as predicted by a computer graphics technique based upon the known crystallographic structure of bovine γ_{II} -crystallin. Thus, gene structure has been related to protein structure in the β/γ -superfamily of proteins.

5. The mouse β -crystallin polypeptide indicates that this protein contains a unique, extremely hydrophobic extension at its N-terminus, raising the possibility that this polypeptide interacts with the cell membrane.

6. Four different mouse γ -crystallin cDNAs have been cloned. Each codes for a different γ -crystallin polypeptide as judged by restriction mapping and hybrid-selection for translation. The γ -crystallins thus appear to form a closely related family of genes.

7. Four different chicken β -crystallin cDNAs have been cloned. Like the mouse γ -crystallin cDNAs, each codes for a different β -crystallin polypeptide. Southern blot analysis indicates that each cDNA is encoded in a separate gene. Developmental studies of β -crystallin synthesis showed that one of these genes is expressed selectively in elongating lens cells.

8. Full-length (or nearly so) cDNAs have been constructed for chicken δ -crystallin. Much of the primary structure for δ -crystallin has been determined.

9. A complete duck δ -crystallin gene containing 14 introns has been cloned. Its structure appears very similar to that of the chicken. The exons of the chicken and duck δ -crystallin gene cross hybridize. These data indicate that the δ -crystallin gene structure has been well conserved throughout evolution.

10. A developmental study employing a cloned δ -crystallin cDNA showed that δ -crystallin mRNA is lost from the lens between the third and fifth month after hatching. This indicates that δ -crystallin gene shut-off, as well as δ -crystallin gene expression, may be studied in vivo.

11. Investigations on nuclear RNAs of the embryonic chicken lens have shown that the intervening sequences of both δ -crystallin genes are transcribed, and that the processing intermediates are not found in equimolar amounts.

12. The mRNA for a major 27K β -crystallin polypeptide was found to be deficient in the Philly mouse cataract. This is the earliest lesion reported yet for the Philly lens and points to a transcriptional or posttranscriptional developmental defect in this hereditary cataract.

13. Turtle δ -crystallin has been shown to have a similar subunit and secondary structure to other species, but displays differences in tertiary structure. In addition evidence has been provided for a new crystallin in the turtle lens.

Significance to Biomedical Research and the Program of the Institute:
The lens crystallins are a family of evolutionarily conserved proteins that are differentially expressed in the developing lens and are responsible for lens transparency. Understanding the structure, function and evolution of these protein families and their genes contribute to our knowledge of embryonic development, eukaryotic gene expression, cell differentiation, molecular evolution, the visual system and disease (in particular, cataract).

Proposed Course: The following studies are in progress or proposed for FY 1983.

1. Further analysis of the mouse α - and β -crystallin genes.
2. Identification of the genetic defect responsible for the deficiency of the β -crystallin mRNA in the Philly mouse lens.
3. Completion of the cDNA sequence of chicken δ -crystallin.
4. Sequence analysis of the chicken and duck δ -crystallin gene.
5. Expression experiments for mouse β - and avian δ -crystallin genes. Initial test will be conducted utilizing in vivo transformation and/or injection methods.
6. Further analysis of the putative, new crystallin from the turtle lens.

NEI Research Program: Cataract--The Norman Lens

Publications:

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Inana G, Shinohara T, Maizel JV Jr, Piatigorsky J: Evolution and diversity of crystallins. J Biol Chem 257:9064-9071, 1982.

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Carper D, Shinohara T, Piatigorsky J, Kinoshita JH: Deficiency of functional messenger RNA for a developmentally regulated β -crystallin polypeptide in a hereditary cataract. Science 217:463-464, 1982.

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Jones RE, Bhat SP, Shinohara T, Piatigorsky J: New directions for cataract research: Use of recombinant DNA technology, in Sears ML, (Ed.): New Directions in Ophthalmic Research. New Haven, Connecticut, Yale University Press, 1981, pp. 89-107.

Jones RE, DeFeo D, Piatigorsky J: Transcription and site-specific hypo-methylation of the δ -crystallin genes in the embryonic chicken lens. J Biol Chem 256:8172-8176, 1981.

Piatigorsky J: Lens research: More than meets the eye. Cell 27:233-235, 1981.

Williams LA, Piatigorsky J, Horwitz J: Structural features of δ -crystallin of turtle lens. Biochim Biophys Acta (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00132-01 LMDB

PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Molecular Biology of Photopigments

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Toshimichi Shinohara	Ph.D.	Biologist	LMDB NEI
Others:	Graeme J. Wistow	Ph.D.	Fogarty Fellow	LMDB NEI
	James P. Alligood	B.S.	Biologist	LVR NEI

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Molecular and Developmental Biology

SECTION

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.25	PROFESSIONAL: 0.75	OTHER: 0.5
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CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The elucidation of the structure, organization and function of photopigments (rhodopsin and color pigments) and their genes is of fundamental importance. Molecular cloning is a powerful method for studying the function and dysfunction of these visual pigments. Retinal mRNAs from rat, bovine and human eyes were extracted and purified. cDNAs made from the purified mRNAs were cloned by the G-C tailing procedure in the bacterial plasmid pBR322. Rat and bovine recombinant cDNAs libraries were established and are being screened for opsin sequences by hybrid selection and cell-free translation. The nature of the retinal binding site is also under investigation. Retinal binding through an apparent Schiff base linkage to calf γ -crystallin was found.

Project Description:

Objectives: The objectives of this project are 1) extract the retinal RNAs from rat, bovine and human eyes and purify the mRNAs, 2) construct the retinal recombinant cDNA libraries, 3) isolate recombinant cDNAs containing opsin sequences, 4) determine the cDNA sequences which can be used to deduce the primary sequence of opsin, 5) isolate opsin genomic DNAs, 6) analyze polypeptide and DNA sequences by computer, and 7) study the retinal binding site to opsin.

Methods Employed: mRNA was purified from rat, bovine and human retina by phenol extraction followed by oligo (dT)-cellulose chromatography. Retinal mRNAs were reverse transcribed, made double-stranded with *E. coli* DNA polymerase I, and tailed with deoxycytidine at the 3' ends by using terminal transferase. The tailed double-stranded cDNAs were hybridized to pBR322 DNA cut by *Pst* I, tailed with deoxyguanosine at the 3' end and used to transform *E. coli* *M1061*. Transformed colonies were screened by the colony hybridization procedure using (³²P) cDNAs synthesized from the retinal mRNAs. The recombinant plasmids were identified by the hybridization selection and translation method.

Major Findings: 1.) cDNA libraries for bovine and rat retina have been established and are being screened for opsin sequences. 2.) Retinal binding to calf γ -crystallin through an apparent Schiff base linkage has been observed. We anticipate that this will be useful for understanding retinal binding to opsin since the crystallographic structure and sequence of γ -crystallin are known.

Significance to Biomedical Research and the Program of the Institute: The elucidation of the molecular mechanism of visual excitation (transduction) is of fundamental importance in eye research. The photopigments present in rod (rhodopsin) and cone cell (color pigments) are responsible for this transduction; therefore, it is essential to know the structure, organization and function of these proteins and their genes.

Recombinant DNA technology provides new and fast methods for the study of photopigments. Direct analysis of cloned cDNAs and their genes will provide knowledge of protein primary sequence, gene structure and differential gene activity for normal and abnormal photopigments.

Proposed Course: This project will be continued. Determination of rat, bovine and human opsin cDNA sequences and screening of rat opsin gene(s) from the genomic library will be performed. The DNA sequences and polypeptide sequences will be analyzed by computer.

NEI Research Program: Retinal and Choroidal Diseases--Photoreceptors, Visual Pigments, and Phototransduction

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00128-01 LMDB

PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Isolation of Type IV Collagen Specific cDNA Clones to Study Eye Morphogenesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Gabriel Vogeli	Ph.D.	Visiting Scientist	LMDB NEI
Other:	David Yang	Ph.D.	Guest Worker	LMDB NEI
	Adriel Bettleheim		Summer Student	LMDB NEI

COOPERATING UNITS (if any)

Mark Sobel, Ph.D., and Marion Young, Ph.D., Laboratory of Developmental Biology and Anomalies, National Institute of Dental Research

LAB/BRANCH

Laboratory of Molecular and Developmental Biology

SECTION

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

1.3

PROFESSIONAL:

1.1

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

 (a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER (a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

To study the molecular biology of eye development, I am isolating cDNA clones for high molecular weight lens proteins. Type IV collagen is a major constituent of the lens capsule. Its subunits are coded for by a large messenger RNA of at least 5000 nucleotides. I have constructed a cDNA library in the plasmid pBR322 using high molecular weight RNA (greater than 4500 nucleotides in length) from total chick embryos. This library is screened with *in vitro* labelled high molecular weight RNA from embryonal chick lenses. Thirty-five cDNA clones from a total of 4200 clones hybridized specifically with the labelled lens RNA. By counter selection with RNA's from different tissues that contain different types of collagen (RNA from cartilage and RNA from chorion-allantois membranes), two prospective type IV collagen specific cDNA clones were selected. These clones are being further analyzed.

Project Description:

Objectives: The development of the embryonic chick eye is very well characterized at the microscopic level. During lens formation the epithelia of cornea and lens are separated from each other and later follow remarkably different developmental pathways. The lens basement membrane will increase in size during the life of the organism, whereas the corneal basement membrane will accumulate other types of collagen in a highly ordered fashion (Coulombre, et al). To analyze at the molecular level the events during lens and cornea formation, the appropriate molecular probes are being isolated. cDNA clones for structural proteins of the lens, like type IV collagen, have to be generated in order to analyze the regulation of genes coding for these proteins.

Methods Employed: Standard techniques of recombinant DNA technology are used to isolate cDNA clones specific for type IV collagen. Since type IV collagen molecules have a molecular weight of around 180,000 to 200,000 daltons, mRNA coding for this molecules have to be at least 5000 nucleotides long. Therefore, the cDNA library was constructed with high molecular weight RNA. Since basement membranes are found throughout the body of animals, mRNA for the construction of the cDNA library was isolated from total chick embryos. The cDNA library was then screened with in vitro labelled high molecular weight RNA from the lens and from the chorion-allantois membranes, which are also rich in type IV collagen. Positive clones were grown up and analyzed by restriction mapping and DNA sequencing.

Major Findings: Two prospective type IV cDNA clones have been isolated by screening with in vitro labelled high MW RNA from different sources. Thirty-five cDNA clones out of a total of 4200 clones hybridized specifically with lens RNA. When the same library was used to screen for type II collagen clones it was noticed that 23 of the 35 lens specific clones also hybridized with cartilage RNA. These 23 clones must be specific for proteins which are common to the tissues of cartilage and lens. When the remaining 12 lens specific clones were hybridized with RNA from chorion-allantois membranes, 4 clones were found to hybridize to both RNA's. Since the chorion-allantois membranes are rich in type IV collagen, these four clones, are good candidates for type IV collagen clones.

Significance to Biomedical Research and the Program of the Institute: The regulation of basement membrane synthesis is one of the crucial events during the morphogenesis of many organs, including the eye. The biosynthesis of basement membranes is severely affected in the course of diabetes. Diabetic induced alterations in the basement membranes of epithelia can lead to defects in many tissues including the tissues of the eye. Insights into the molecular basis of basement membrane biosynthesis is important for the understanding of normal development and the course of events during diseases like diabetes.

Proposed Course: The two isolated clones will be further analyzed to determine for which protein they are coding. Hybrid selected mRNA will be translated and the identity of the protein determined by antibody precipitation. Since the clones contain only relative small inserts, a new cDNA library will be made. In addition the genomic library in Charon 4A will be screened to isolate the gene.

Project No. Z01 EY 00128-01 LMDB

NEI Research Program: Cataract--The Normal Lens

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00127-06 LMDB

PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Plasma Membrane Composition and Biosynthesis in Chick Lens Fibers and Epithelia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Peggy Zelenka	Ph.D.	Geneticist	LMDB	NEI
Other:	Ngoc-Diep Vu	Ph.D.	Staff Fellow	LMDB	NEI

COOPERATING UNITS (if any)

David Beebe, Ph.D., Ass't Professor, USUHS

LAB/BRANCH

Laboratory of Molecular and Developmental Biology

SECTION

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

2.05

PROFESSIONAL:

2.05

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS

(b) HUMAN TISSUES

(c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project seeks to determine whether the regulation of lens fiber differentiation and maturity is associated with alterations in the plasma membrane. The principal lipid and protein components of embryonic and adult chicken lenses have been identified and their metabolism is being investigated. The relationships between phospholipid metabolism and differentiation have been studied in vivo and in vitro using isotopic labeling techniques and computer modeling. A transient increase in the transmethylation of phosphatidylethanolamine has been shown to be an initial event in lens fiber cell formation. In addition, rapid turnover of phosphatidylinositol, which occurs in lens epithelial cells, ceases abruptly following the initiation of lens fiber formation both in vivo and in vitro.

Project Description:

Objectives: The objectives of this project are: (a) to characterize the principal lipid and protein components of plasma membranes from embryonic chick lens fibers and lens epithelial cells; (b) to determine whether the differentiation of lens epithelial cells into lens fibers is accompanied by changes in membrane composition; (c) to learn whether the differentiation of lens epithelial cells into lens fibers is accompanied by changes in the metabolism of lens plasma membrane constituents; and (d) to establish the functional significance of any changes in membrane composition or metabolism.

Methods Employed: In vitro studies of lens phospholipid metabolism employ cultured explants of lens epithelia from 6-day-old or 19-day-old embryonic chicks. Various culture conditions are used to maintain the cells in an epithelial state or to permit their differentiation into lens fibers. Phospholipids are labeled with ^{32}P -orthophosphate, ^3H -glycerol, ^3H -methionine, or ^3H -labeled fatty acids. Drugs which interfere with phospholipid and arachidonic acid metabolism are added to the culture medium to test their effect on differentiation. Labeled phospholipids are analyzed by thin layer chromatography and scintillation counting, while fatty acids and their metabolites are analyzed by high performance liquid chromatography (HPLC).

Precursors of phospholipid synthesis are isolated by anion exchange chromatography, and their concentrations are determined by means of a colorimetric assay for phosphate. Knowledge of precursor concentrations allows calculation of precursor specific radioactivities, making possible determinations of rates of synthesis and degradation of individual phospholipids by computer modeling.

Major Findings: A transient increase in the transmethylation of phosphatidylethanolamine to phosphatidylcholine has been shown to be an initial event in lens fiber cell differentiation. The accumulation of the methylated product is due to increased activity of the methyltransferases rather than to decreased activity of phospholipase A, the enzyme which degrades the methylated product. Inhibition of phospholipid methylation by 3-deazaadenosine also inhibits the cellular elongation that characterizes lens fiber formation. The concentration of 3-deazaadenosine which produces half-maximal inhibition of both phospholipid methylation and elongation is $3\mu\text{M}$. This concentration of the drug only slightly inhibits methylation of proteins, neutral lipids, and nucleic acids. Thus, the transient stimulation of phospholipid methylation, which occurs within 6 sec of the onset of in vitro lens fiber formation, seems to be a required step in the subsequent differentiation of the cells.

In vitro studies using cultured, embryonic chicken lens epithelia have confirmed the earlier in vivo observation that a marked decrease in the rate of phosphatidylinositol turnover accompanies lens fiber cell formation. The in vitro studies have further shown that rapid turnover of phosphatidyl-
inositol occurs in cells of the central region of the lens epithelium, and involves only the phosphoinositol headgroup of the molecule. The activity or amount of the phospholipase C responsible for phosphatidylinositol degradation

decreases within the first few hours following the initiation of *in vitro* lens fiber cell differentiation by fetal calf serum, insulin, or vitreous humor. Although the activity of this enzyme in the epithelial cells is stimulated by increases in intracellular calcium, the inactivation of the enzyme following the onset of fiber formation is not regulated by a decrease in intracellular calcium.

Drugs which inhibit various enzymes involved in the release and metabolism of arachidonic acid have been shown to promote *in vitro* lens cell elongation, even in the absence of other stimulating agents, such as fetal calf serum. All phospholipase A inhibitors tested had this effect, as did two inhibitors of the lipoxygenase pathway of arachidonic acid metabolism. Inhibitors of the cyclooxygenase pathway, which leads to the production of prostaglandins, prostacyclin, and thromboxanes, had no effect on lens cell elongation. These data suggest that lipoxygenase metabolites of arachidonic acid may play a role in regulating the elongation which is characteristic of lens fiber cell formation.

Significance to Biomedical Research and the Program of the Institute: The plasma membranes of lens cells appear to play important roles in normal development and function of the lens. In addition, they are centrally involved in the genesis and development of several varieties of lens cataract. Despite the widely recognized and important functions of these membranes, work on their composition, turnover, and development has begun only recently. This project focuses on changes in lens cell membranes which are associated with lens fiber differentiation. These results should have broad application in understanding normal lens differentiation and morphogenesis and in attempts to establish etiologies for several types of cataract.

Proposed Course: This project will be continued. Experiments in progress to study the metabolism of phospholipids other than phosphatidylinositol, both *in vivo* and *in vitro*, will be continued. These experiments will determine whether phosphatidylinositol turnover has a unique role in the lens epithelium or is part of a more general phenomenon.

Techniques will be devised for measuring calcium fluxes and calcium concentrations in cultured explants of embryonic chicken lens epithelia, in order to test whether the observed changes in phosphatidylinositol turnover are correlated with changes in the calcium permeability of the plasma membrane. Such correlations have been found in other cell types.

An attempt will be made to analyse the metabolites of arachidonic acid produced by embryonic chicken lens epithelia *in vitro*, using high performance liquid chromatography, in order to study the possible role of these metabolites in the regulation of lens fiber cell formation.

NEI Research Program: Cataract--The Normal Lens

Publications:

Zelenka PS, Jernigan HM Jr: Phosphorylcholine and phosphorylethanolamine concentrations in the lens. Exp Eye Res 34:209-217, 1982.

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Vu ND, Chepko G, Zelenka P: Decreased turnover of phosphatidylinositol accompanies in vitro differentiation of embryonic chicken lens epithelial cells into lens fibers. Biochim Biophys Acta (in press).

Laboratory of Sensorimotor Research

ANNUAL REPORT
NATIONAL EYE INSTITUTE
October 1, 1981 - September 30, 1982

REPORT OF THE CHIEF, LABORATORY OF SENSORIMOTOR RESEARCH
Robert H. Wurtz, Ph.D.

During this fourth year since the organization of the Laboratory of Sensorimotor Research, part of the facilities for the laboratory in Building 10C became available. Research laboratory space was first occupied in January; the animal facility was completed in July. A substantial part of the effort of the laboratory was devoted to constructing new laboratories and moving established laboratories to Building 10.

The experimental projects within the laboratory are summarized in three groups according to the visual-oculomotor system studied: control of saccadic eye movements, control of smooth pursuit eye movements, and plasticity of visual-motor control. This grouping is not all-inclusive as will be evident from the individual project reports, but is intended to emphasize highlights of research during this past year. The experiments range from studies of anatomical connections and cellular activity in the brains of monkey to the normal and pathological oculomotor functions in man.

The first group of experiments concentrated on the initiation of saccadic eye movements, those that jump the eye quickly from one fixation point to another. Previous work in this laboratory showed that neurons within an area of the frontal lobe, the frontal eye fields, have visual receptive fields that show an enhanced response when a stimulus is used as the target for saccadic eye movements. In addition, microstimulation of this area of cortex evoked eye movements to parts of the visual field covered by the visual receptive fields of neurons at the stimulated sites. Experiments have now demonstrated that the receptive fields and the eye movements evoked by stimulation have a topographic organization within the frontal eye fields so that large eye movements are related to the most dorsomedial area, small eye movements are related to the most ventrolateral area. Furthermore, anatomical experiments show that the areas related to large and small eye movements have prominent connections to the areas of the superior colliculus related to large and small eye movements, respectively. In addition, some cells discharge in relation to eye movements evoked by auditory stimuli, suggesting that cells in this area act as if they were constructing a supramodal map of eye movements. These experiments further establish the frontal eye fields as the cerebral cortical area most directly involved in the initiation of saccadic eye movements.

One possible output pathway leading from the frontal cortex, including the frontal eye fields, passes through the caudate nucleus of the basal ganglia to the substantia nigra and thence to the superior colliculus. Work in the laboratory in the previous two years has shown that the cellular activity in the substantia nigra is related to visual processing and the initiation of saccadic eye movements. A type of cellular response has now been identified which is modulated by fixation of gaze. One type of these fixation-related responses occurs to spots of light in the visual field only while the monkey is not looking at a fixation spot. Control experiments established that it is not the act of fixation that suppresses the response of

these cells to other spots of light, but rather the visual stimulus itself. A second type of response related to the monkey's fixation of gaze is the response to the offset of the spot being fixated. In order for this response to be observed the monkey must be fixating on a spot of light and no other spot of light can be present in the visual field. The significance of these responses can best be appreciated by realizing that they occur only at two times: with the appearance of a spot of light that the monkey is going to fixate and with the disappearance of a spot of light that the monkey has been fixating. These cells might facilitate the onset and termination of a series of visually-guided saccades.

The superior colliculus is the area to which many of the fibers in substantia nigra and frontal eye fields project. Anatomical experiments this year show that three subcortical structures are the most prominent source of projections to the superior colliculus: substantia nigra, the parabigeminal nucleus, and the rostral mesencephalic reticular formation. The cortical area with the most prominent projection to the superior colliculus is the frontal eye fields. These projections are selectively directed toward the intermediate layers of the superior colliculus where cells related to the initiation of saccadic eye movement are located.

The pathway leading from the superficial layers of the superior colliculus, which are related to visual processing, to the cerebral cortex passes through an area of the thalamus, the pulvinar nuclei. The anatomical divisions of this area have only recently been identified, and work in the laboratory this year has characterized the activity of cells in four subdivisions of the pulvinar: the inferior pulvinar, the alpha division of the lateral pulvinar, the beta portion of the lateral pulvinar, and the medial pulvinar. Each of these subdivisions has been shown to have different relationships to stimulus movement and to the initiation of saccadic eye movements. In particular, it is worth noting that an area that has been thought of largely as being related to visual processing has cells that have saccade-related activity. These responses can be seen with visually-guided saccadic eye movements but also with eye movements made spontaneously in total darkness. Cells with these characteristics are common in the beta subdivision of the lateral pulvinar.

The second group of experiments centered on the pursuit system that moves the eye in order to achieve the velocity of a moving target and allows man and monkey to fixate on this moving target. The neural basis of pursuit movements is even less well understood than is that for the initiation of saccadic eye movements, and several experiments this year have been devoted to analyzing this system. The visual cells that logically would feed into a pursuit system should be related to velocity of a moving stimulus and the direction of a moving stimulus. Cells with these characteristics have been identified in the middle temporal area of monkey cerebral cortex, and many cells in this area discharge during pursuit eye movements. Neurons in an area adjacent to the middle temporal area discharge when the monkey makes smooth eye movements in a particular direction, and these cells continue to discharge even when the pursuit is made in total darkness. The response is clearly generated by the motor task and not by spurious visual stimulation during the task. While there is no precise explanation for these responses, it seems likely that these neurons would be useful during the maintenance of pursuit eye movements or in dealing with the perceptual consequences of these eye movements. In

addition, at an anatomical level, this area and several others have been histologically delineated from surrounding tissue for the first time by the use of myelin stains. This new anatomical characterization allows a closer correlation between anatomical area, cell activity, and behavior of the monkey.

Ablation of the primary visual cortical area, striate cortex, has been investigated in relation to the initiation and maintenance of pursuit eye movements. While monkeys with a large ablation of one striate cortex are able to make saccadic eye movements to stationary targets, they cannot use stimulus velocity or position information in the visual field served by the ablated cortex to generate a smooth pursuit eye movement. Nor can these animals make accurate saccadic eye movements to stimuli that are moving in this visual field since they cannot compensate for target velocity. These experiments indicate that striate cortex is necessary for the normal translation of stimulus velocity into eye movement velocity.

These studies on the neural basis of the saccadic and pursuit systems are not only essential for an understanding of how the brain generates these movements but for an understanding of the basic mechanisms by which the brain converts sensory information into movement programs. An understanding of these mechanisms also aids in the diagnosis of the deficits observed in patients since the visual and oculomotor behavior of man and monkey are similar.

The third group of experiments concerns the plasticity of visual-motor control and concentrates on the abilities of man. This plasticity has been investigated first in the relationship between vergence eye movement and accommodation systems. Transfer of fixation between targets at different distances involves changes in both vergence of the eyes and accommodation of the lens which act to eliminate disparity and blur, respectively. Blur as a stimulus to accommodation operates very slowly while disparity as a stimulus to vergence is much quicker. Under normal viewing conditions, however, changes in accommodation usually lag only slightly behind changes in vergence because the two systems are cross-coupled. This cross-coupling can be revealed using pinhole viewing conditions where blur cues are eliminated. Disparity-induced changes in vergence automatically elicit changes in accommodation. This is an open-loop system, that is, without immediate feedback, and as with all open-loop systems, there is a calibration problem which leads to the question of how the system knows by how much to alter accommodation for a given change in vergence.

This question was answered by having human subjects wear magnifying or reducing spectacles for 30 minutes and then checking the relationship between vergence and accommodation. Challenged with optical devices that increased the apparent separation of the two lines of sight, the gain of the vergence-accommodation response showed a decrease that was appropriate for improving the rapid focusing of the eyes during transfer of fixation. This strongly suggests that the normal coupling between vergence and accommodation is subject to adaptive regulation. On the other hand, a decrease in the apparent separation of the two lines of sight did not decrease the gain of the response. These observations are particularly important since by far the most common clinical disorders of eye movement involve misalignments of the two eyes with respect to one another and these experiments show the extent of the adaptive capability of the system.

Plasticity in visual-motor coordination was also revealed in a study of a human patient scheduled for strabismus surgery. This patient had a lateral rectus palsy of one side which resulted in an inability to abduct one eye. By covering the normal eye with a patch, it was possible to force the patient to view the world through the eye that had inadequate muscular control. The saccadic system and the vestibular-ocular reflex altered their innervation to compensate for the peripheral weakness as had been shown previously. What is new and surprising is that the smooth pursuit system also changed adaptively by increasing its innervation. The adaptive changes of the vestibular, saccadic, and pursuit movements depended on both orbital position and direction and compensated for changes in orbital mechanics. In addition to showing adaptive changes in the pursuit system, this study attempts to determine to what extent central adaptive mechanisms are involved in strabismus disorders and in the patient's recovery from strabismus surgery. Such studies may result in new diagnostic tests, possibly with some ability to predict the success of strabismus surgery.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00047-04 LSR																								
PERIOD COVERED October 1, 1981, to September 30, 1982																											
TITLE OF PROJECT (80 characters or less) Visual Processing in Brains following Cortical Ablation																											
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																											
<table> <tr> <td>PI:</td> <td>Michael E. Goldberg</td> <td>M.D.</td> <td>Chief, Section on Neuro-Ophthalmologic Mechanisms</td> <td>LSR</td> <td>NEI</td> </tr> <tr> <td>Other:</td> <td>Charles J. Bruce</td> <td>Ph.D.</td> <td>Senior Staff Fellow</td> <td>LSR</td> <td>NEI</td> </tr> <tr> <td></td> <td>Leslie G. Ungerleider</td> <td>Ph.D.</td> <td>Senior Staff Fellow</td> <td>LN</td> <td>NIMH</td> </tr> <tr> <td></td> <td>Mortimer Mishkin</td> <td>Ph.D.</td> <td>Research Physiologist</td> <td>LN</td> <td>NIMH</td> </tr> </table>				PI:	Michael E. Goldberg	M.D.	Chief, Section on Neuro-Ophthalmologic Mechanisms	LSR	NEI	Other:	Charles J. Bruce	Ph.D.	Senior Staff Fellow	LSR	NEI		Leslie G. Ungerleider	Ph.D.	Senior Staff Fellow	LN	NIMH		Mortimer Mishkin	Ph.D.	Research Physiologist	LN	NIMH
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COOPERATING UNITS (if any) Department of Neurology, Georgetown University School of Medicine Laboratory of Neuropsychology, NIMH																											
LAB/BRANCH Laboratory of Sensorimotor Research																											
SECTION Neuro-Ophthalmologic Mechanisms Section																											
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205																											
TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.5	OTHER: 0.0																									
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<p>SUMMARY OF WORK (200 words or less - underline keywords)</p> <p>The <u>striate cortex</u> of one <u>cerebral hemisphere</u> of a <u>rhesus monkey</u> is removed surgically under direct vision. The monkeys are allowed to recover from the effects of surgery in a normally lit environment. The monkeys are then trained on a series of tasks requiring <u>visual perception</u> and <u>visually-guided eye movements</u>. They are then prepared for chronic neurophysiological recording and for eye position recording. The activity of <u>single neurons</u> in the <u>frontal eye fields</u> and <u>posterior parietal cortex</u> both ipsilateral and contralateral to the lesion is studied. The monkey's oculomotor capacity is studied quantitatively. The frontal eye fields are much less visually responsive than normal although eye movements can still be evoked at low threshold from the silent area. The animals can make normal <u>saccadic eye movements</u> to stationary targets in the blind field although they cannot make accurate saccades to moving stimuli. They cannot use stimulus velocity or position information in the contralateral field to generate a <u>smooth pursuit eye movement</u>. These data indicate that the striate cortex is necessary for the normal functioning of visual association areas, including those involved in oculomotor processing.</p>																											

Project Description:

Objectives: Information from the retina can reach the parietal and frontal cortex in two ways: first, via the lateral geniculate nucleus of the thalamus, then through the striate and prestriate cortex; second, via the superior colliculus, then through posterior thalamic nuclei. These pathways interact: the striate cortex projects to the superior colliculus and the pulvinar of the thalamus, and the pulvinar projects to the prestriate cortex. Nonetheless, these areas are each capable of contributing to visual behavior in the absence of the other. Even in humans, where it was traditionally thought that striate damage led to total visual impairment, it has now become clear that there is some residual visual function in the absence of striate cortex which can be accessed using forced choice or nonverbal methods of evaluation. Since parietal and frontal cortex should receive the subcortical visual pathway in the absence of striate cortex, it was of interest to see if this area had enough visual processing to support the behavior found in the presence of striate lesions. Initial work in this laboratory showed that this residual visual processing could be performed in the cerebral cortex by parietal neurons that are visually responsive even in the absence of striate cortex. This visual activity was limited to a small area of the parietal cortex, but within this area the area seemed quite normal. Histological analysis found this striate independent function to be present in at least two discrete areas of parietal cortex: one on the dorsum of the inferior parietal lobule at the interparietal sulcus and one in the posterior part of the inferior parietal lobule in the superior temporal sulcus. Recent work on this project has concentrated on examining the residual function of the frontal eye fields, and a quantitative analysis of the oculomotor properties strategies employed by animals with unilateral striate cortex lesions.

Methods Employed: Rhesus monkeys underwent unilateral striate ablation. After recovery from surgery they were trained to perform a number of visuomotor tasks including visual fixation, visually-guided smooth pursuit, and saccadic eye movements. The monkeys were implanted with magnetic search coils in order to measure accurately the eye position in space. A digital computer was used for on-line eye position and velocity analysis and stimulus presentation.

Major Findings: The frontal eye fields ipsilateral to the striate lesion are practically devoid of visual activity. Saccades can be evoked from this region by electrical stimulation at currents comparably low to those effective in normal monkeys, but we could not find any comparable activity discharging before visually-guided eye movements.

Animals with striate lesions are capable of saccadic eye movements of normal amplitude and velocity into the contralateral visual field no longer represented by striate cortex. However, they must make these saccades to static targets. If the targets are moving, the monkeys cannot compensate for the ongoing movement by predicting where the target will be at the end of the saccade. Instead, they make an eye movement to where the target was at some point at the beginning of the saccade processing. Thus although they can locate a moving target, they treat it as if it were not moving.

Monkeys with ipsilateral lesions can also make perfectly adequate smooth pursuit eye movements as long as the stimulus remains on the fovea or in the ipsilateral visual field. Several techniques were used to force the animal to use the hemianopic field. In one experiment the animal was required to track a vertically moving target. At some point after the track was established the computer driven stimulus presentation system stepped the stimulus into one hemifield, and maintained it there by feeding back eye position and adding it to stimulus position. This technique effectively forces the stimulus to stay in the chosen field. The downward pursuit velocity rapidly drops to zero when the image steps into the field contralateral the lesion, but not when the computer steps into the ipsilateral field. In a second experiment the animal was required to track a sinusoidally moving stimulus which then stepped into the blind field. In this case also the eye velocity behaved as if the animal were pursuing a stimulus that had disappeared.

Significance to Biomedical Research and the Program of the Institute:
These results show that under normal circumstances the oculomotor system gets much of its cortical visual information through the geniculostriate system. Although this information is not necessary for all visual oculomotor processing, since both man and monkey can make eye movements without striate cortex, it clearly is important when the computational problem becomes more complex than discovering merely where a stimulus is. Understanding how the brain parcels out different aspects of the process of visual analysis to different areas will not only further our knowledge of the functions of the normal brain but may also contribute to the development of strategies for the rehabilitation of patients with damage to those brain areas involved in some aspects of vision.

Proposed Course: A further survey of visual responsiveness of frontal and parietal neurons will be undertaken to verify the existence and location of the previously described areas of spared function. The oculomotor capability, including optokinetic nystagmus, of animals with striate lesions will be further analyzed. Since it has been reported that monkeys with bilateral lesions of the occipital lobe are capable of making perfect smooth pursuit movements with foveal stimuli, animals with serial striate ablations will be studied to see if peripheral smooth pursuit can be restored by the second striate lesion. A clinical protocol to study visual and oculomotor function in hemianopic patients will be initiated.

NEI Research Program: Strabismus, Amblyopia, and Visual Processing--Visual Processing and Amblyopia (Disorders--Sensory Neuro-Ophthalmic Disorders)

Publications: None



SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00049-04 LSR

PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Cerebral Cortical Mechanisms for Eye Movements and Visual Attention

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Michael E. Goldberg M.D. Chief, Section on LSR NEI
Neuro-Ophthalmologic
Mechanisms
Other: Charles J. Bruce Ph.D. Senior Staff Fellow LSR NEI

COOPERATING UNITS (if any)

Department of Neurology, Georgetown University School of Medicine
Department of Anatomy, Howard University

LAB/BRANCH

Laboratory of Sensorimotor Research

SECTION

Neuro-Ophthalmologic Mechanisms Section

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies are being conducted to determine the mechanisms through which the frontal eye fields of the cerebral cortex exert control over eye movements and visual attention in the monkey. Single-cell recordings are made while the monkeys perform a series of visual tasks involving eye movements or visual fixation. Previous work in this laboratory has found a neural mechanism for the generation of visually-guided eye movements in frontal eye fields. The frontal eye fields have a topographic map, so that cells associated with large eye movements are located dorsomedially in the frontal eye fields, and cells subserving small eye movements are located ventrolaterally. Cells in the area subserving the largest eye movements have auditory as well as or instead of visual responses. Cells near the region subserving small saccades discharge during smooth pursuit eye movements, and electrical stimulation here evokes smooth eye movements. The frontal eye fields have a topographic projection to the area of the superior colliculus subserving eye movements, so that the area related to large eye movements projects to the caudal superior colliculus, and the area related to small eye movements projects to the rostral superior colliculus.

Project Description:

Objectives: Previous work in this laboratory established that of the two visual association areas most likely to be involved in the visual initiation of eye movements, the posterior parietal cortex and the frontal eye fields, the latter was by far the better candidate. In this area, neurons with visual receptive fields were shown to give enhanced discharges before eye movements to the stimulus in their receptive field, but not when the same stimulus was used in other kinds of behavior. Conversely, in the parietal cortex, neurons which gave enhanced responses were shown to yield these responses whenever the animal attended to the target, not only when it made a saccadic eye movement to it. In addition, low threshold microstimulation through the recording microelectrode revealed that the eye movements evoked by this stimulation were predicted by the visual receptive field of the neurons at the stimulated site. Subsequent investigation revealed that a significant population of frontal neurons discharged not only with visually-guided eye movements but also in association with eye movements to remembered spots of light in total darkness. The cells discharged much less, if at all, before spontaneous eye movements in total darkness or light. Thus it was clear that not all eye movements were associated with frontal activity, but only those eye movements which were associated with purposive behavior. Although eye movements are predominantly visually guided, they need not be. In particular, eye movements can be made to auditory or somatosensory stimuli. Given these data, it was decided to investigate in greater detail the oculomotor and sensory properties of the frontal eye fields in order to gain a greater understanding of the cortical processing involved in the generation of eye movements. In addition, a study was undertaken to establish the topographic limits of the frontal eye fields, and to see if a topographic organization was present in the projections of the frontal eye fields.

Methods Employed: A digital computer was used for behavioral control, data acquisition, and on- and off-line analysis of monkey behavior, eye movement, and neuronal discharge time patterns. Monkeys were trained on a series of visuomotor tasks, including visual fixation, saccadic eye movements to visual and auditory stimuli, eye movements to remembered points, and smooth pursuit eye movements. Movements to remembered targets and successive saccadic activity of single units were measured during these tasks. Eye movements were measured using the magnetic search coil technique, so that accurate quantitative measures of eye position and velocity could be obtained. A computerized system was developed so that eye movements of different targets could be presented in random and pseudorandom fashion. Several untrained monkeys were prepared for semichronic experiments in which large areas of the frontal cortex could be explored under direct vision in order to find low threshold areas and make a map of eye movements evoked by stimulation. These areas could then be used as the sites for small isotope injections in order to correlate the size of eye movement evoked by electrical stimulation at a given point with the projection of that point.

Major Findings: The frontal eye fields contain a series of visually responsive neurons, and also neurons which discharge before saccades relevant to the animal's behavior. The receptive fields and eye movements evoked by

stimulation have a topographic organization, so that large eye movements are located in the dorsomedial frontal eye fields, and small eye movements are located ventrolaterally. The area of the frontal eye fields associated with large eye movements has cells which respond to auditory stimuli located in the contralateral auditory field. Some cells respond more briskly to auditorily evoked eye movements than to passive auditory stimuli. Some cells respond to visual and auditory stimuli located in the same region, as if the cells were constructing a supramodal map of eye movement space.

The area of the frontal eye fields associated with small eye movements blends into an area associated with smooth pursuit eye movements. This area has cells which discharge during smooth pursuit movements, and electrical microstimulation causes the monkey to develop a smooth pursuit movement for the duration of the stimulus. Eye movement velocity analysis reveals that the monkey is maintaining a true smooth pursuit movement although it also makes a few small saccades.

Electrical stimulation evokes eye movements at low current thresholds in the frontal eye fields. Mapping experiments under several sets of conditions were performed to determine the topological organization of eye movements within the frontal eye fields. Large eye movements are located in the most dorsomedial part of the frontal eye fields, and small eye movements in the most ventrolateral area. The entire cortical area of the frontal eye fields as determined by intracortical microstimulation is limited to the anterior bank of the arcuate sulcus and the immediate adjacent gyral surface, and extends from the most posterior part of the arcuate sulcus for about half a centimeter laterally. This area is much smaller than the frontal eye fields as described in the literature. However, this is the only area in which cells discharging before purposive movements and eye movements evoked by very low (less than 50 μ amperes of current) levels of electrical stimulation can be evoked. The fibers from this low threshold area course anteriorly, and eye movements at low threshold can be evoked from the fiber bundles themselves. Incidental excitation of the fiber bundles may explain why macrostimulation experiments in the past have indicated that the frontal eye fields seemed much larger.

The anatomic projections from the frontal eye fields to the superior colliculus are themselves topologically organized. Cells in the small saccade region project to the intermediate layers of the rostral superior colliculus, where also cells which subserve small eye movements are found. Cells in the large saccade region project to the caudal colliculus, which has cells subserving larger saccades.

Significance to Biomedical Research and the Program of the Institute: The frontal eye fields are important in their own right as the cerebral cortical region concerned with the guidance of eye movements, and also as a cerebral cortical model for a premotor cortex. By understanding the mechanisms by which the frontal eye fields convert visual and auditory information into a signal for an eye movement, we can begin to understand the basic mechanisms by which the brain integrates sensory information into motor programs. We can also begin to understand the mechanisms of the deficits in patients with

lesions in visual-motor control areas, and use this understanding for improved diagnosis and the development of rehabilitative strategies.

Proposed Course: Stimulating electrodes will be placed in several of the areas to which the frontal eye fields project, and the projection properties of physiologically identified frontal eye field neurons will be analyzed. Monkeys with frontal eye field lesions will be studied for their ability to make smooth pursuit movements, saccades to visual and auditory stimuli, saccades to remembered targets, and predictive eye movements.

NEI Research Program: Strabismus, Amblyopia, and Visual Processing--Visual Processing and Amblyopia (Disorders--Sensory Neuro-Ophthalmic Disorders)

Publications:

Bushnell MC, Goldberg ME, Robinson DL: Behavioral enhancement of visual responses in monkey cerebral cortex: I. Modulation in posterior parietal cortex related to selective attention. J Neurophysiol 46:755-772, 1981.

Goldberg ME, Bushnell MC: Behavioral enhancement of visual responses in monkey cerebral cortex: II. Modulation in frontal eye fields specifically related to saccades. J Neurophysiol 46:773-787, 1981.

Goldberg ME: Moving and attending in visual space, in Potegal M (ed): Spatial Behavior: Physiological and Developmental Aspects. New York, Academic Press, 1982, pp 277-300.

Goldberg ME: Iconoclasm avoided: What the single neuron tells the psychologist about the icon. Behav Brain Sci (in press).

Goldberg ME: Studying the neurophysiology of behavior: methods for recording single neurons in awake behaving monkeys, in Barker JL, McKelvey JF (eds): Current Methods in Cellular Neurobiology. New York, Wiley Press (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00113-02 LSR

PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

The Neural Coupling between Vergence Eye Movements and Accommodation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Frederick A. Miles D. Phil. Chief, Oculomotor Control Section LSR NEI

Other: Kenji Kawano M.D., Ph.D. Visiting Scientist LSR NEI

COOPERATING UNITS (if any)

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Johns Hopkins University School of Medicine

LAB/BRANCH

Laboratory of Sensorimotor Research

SECTION

Oculomotor Control Section

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

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(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Transfer of fixation between targets at different viewing distances involves changes in vergence eye movements and accommodation that operate to eliminate disparity and blur, respectively. During pinhole viewing, when blur cues are absent, changes in vergence due to disparity result in linear changes in accommodation: the vergence-accommodation response. Experiments were undertaken to determine whether these open-loop responses are subject to visually-mediated adaptive regulation. Human subjects were fitted with specially made laterally-displacing periscopic spectacles to increase the apparent separation of their two eyes and thereby decrease the required change in accommodation per unit change in vergence to maintain single, clear vision. Thirty minutes of exposure to these spectacles was sufficient to cause large decreases in the accommodative change associated with a unit change in vergence during pinhole viewing. This demonstrates that the coupling between vergence and accommodation is subject to adaptive regulation. Decreasing the apparent separation of the eyes with medially-displacing (cyclopean) spectacles failed to affect the magnitude of the vergence-accommodation response. Thus, the adaptive mechanism shows considerable asymmetry.

Project Description:

Objectives: Transfer of fixation between targets at different distances involves changes in both vergence eye movements and accommodation. Superficially, both of these would seem to result from the operation of negative feedback systems functioning to eliminate disparity and blur, respectively. However, blur as a stimulus to accommodation is very slow, having very long latency, and is only effective in the immediate vicinity of the fovea. By contrast, disparity as a stimulus to vergence is much quicker and is effective over a wide area of the retina. Nonetheless, under normal viewing conditions, changes in accommodation usually only lag slightly behind changes in vergence because the two systems are cross-coupled. This cross-coupling is revealed under pinhole viewing conditions, when blur cues are eliminated, and disparity-induced changes in vergence automatically elicit linear changes in accommodation. This open-loop, vergence-accommodation response is responsible for eliciting a major part of the accommodation response under normal viewing conditions. As with all open-loop responses, there is a calibration problem--how does the system know by how much to alter accommodation for a given change in vergence? Experiments were undertaken to determine whether the vergence-accommodation response was subject to adaptive control by seeing if it could be modified by visual devices that alter the strength of the required coupling between vergence and accommodation.

Methods Employed: The normal coupling between vergence and accommodation was disturbed with specially constructed spectacles that altered the apparent separation of the two lines of sight. The apparent separation was increased by a factor of more than two by means of laterally-displacing binocular periscopes (magnifying spectacles) or decreased to zero with medially-displacing binocular periscopes (cyclopean spectacles). Some control observations were made using base-in and base-out prism spectacles that induce a step alteration in the relationship between vergence and accommodation but, unlike the periscopic spectacles, do not alter the slope of the relationship. Vergence-accommodation responses were measured in human subjects using a haploscope incorporating a laser speckle optometer with Badal lens viewing to determine the accommodative state of the right eye. Both eyes viewed cross-hairs in Maxwellian view, illumination being provided by 1mm point sources to eliminate blur cues. Vergence responses were induced by rotating the left limb of the haploscope around the center of rotation of the left eye, and the associated accommodation induced in the right eye was measured with the optometer.

Major Findings: Vergence-accommodation was expressed as a dimensionless gain parameter equal to the net change in accommodation per unit change in vergence divided by the required change in accommodation to maintain correct focus during that same change in vergence. After wearing the magnifying spectacles for 30 minutes, vergence-accommodation gains were reduced by up to 60%. Neither the cyclopean spectacles nor the base-in prismatic spectacles had any significant effect on the gain of the accommodation-vergence response, though the base-out prism arrangement caused a step decrease in the accommodation coupled to any given vergence state. Thus, challenged with optical devices that increased the apparent separation of the two lines of

sight, the gain of the vergence-accommodation response showed a decrease that was appropriate for improving the rapid focusing of the eyes during the transfer of fixation. This strongly suggests that the normal coupling between vergence and accommodation, as represented by the vergence-accommodation response, is subject to adaptive regulation.

Significance to Biomedical Research and the Program of the Institute: One of the major new developments in the field of oculomotor physiology in the last decade concerns the brain's ability to use visual inputs to regulate its performance. This adaptive capability is important in the achievement of the extraordinary precision that characterizes the neural control of eye movements that is so imperative for clear vision. That the alignment of the two eyes, under the influence of vergence-accommodation, is subject to adaptive regulation is especially interesting since by far the most common clinical disorders of eye movements involve misalignments of the two eyes with respect to one another.

Proposed Course: The adaptive mechanisms maintaining appropriate coupling between the vergence eye movements and accommodation will continue to be the main concern of these studies. Base-out prism adaptation will be used to effectively eliminate vergence-accommodation responses at low levels. This "behavioral lesion" technique will be used to ascertain the role of vergence-accommodation during the transfer of fixation under normal binocular viewing conditions.

NEI Research Program: Strabismus, Amblyopia, and Visual Processing--Ocular Motility and Strabismus (Structure and Function--Vergence and Accommodation)

Publications:

Judge SJ, Miles FA: Gain changes in accommodative vergence induced by alteration of the effective interocular separation, in Fuchs AF, Becker W (eds): Progress in Oculomotor Research. New York, Elsevier North-Holland, 1981, pp 587-594.

Miles FA: Adaptive regulation in the primate oculomotor system. Freiburger Universitatsblatter 74:107-111, 1981.

Miles FA, Lisberger SG: The "error" signals subserving adaptive gain control in the primate vestibulo-ocular reflex. Ann N Y Acad 374:513-525, 1981.

Miles FA, Judge SJ: Optically-induced changes in the neural coupling between vergence eye movements and accommodation in human subjects, in Lennerstrand G, Zee DS, Keller EL (eds): Functional Basis of Ocular Motility Disorders. Oxford, Pergamon (in press).

Miles FA: Adaptive gain control in the vestibulo-ocular reflex, in
Lennérstrand G, Zee DS, Keller EL (eds): Functional Basis of Ocular
Motility Disorders. Oxford, Pergamon (in press).

Miles FA: Plasticity in the transfer of gaze. Trends in Neuroscience (in
press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00182-01 LSR												
PERIOD COVERED October 1, 1981, to September 30, 1982															
TITLE OF PROJECT (80 characters or less) Central Nervous System Compensation for Peripheral Oculomotor Deficits															
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT															
<table> <tr> <td>PI: Lance M. Optican</td> <td>Ph.D.</td> <td>Senior Staff Fellow</td> <td>LSR NEI</td> </tr> <tr> <td>Other: David S. Zee</td> <td>M.D.</td> <td>Visiting Scientist</td> <td>CB NEI</td> </tr> <tr> <td></td> <td>M.D.</td> <td>Senior Staff Fellow</td> <td>CB NEI</td> </tr> </table>				PI: Lance M. Optican	Ph.D.	Senior Staff Fellow	LSR NEI	Other: David S. Zee	M.D.	Visiting Scientist	CB NEI		M.D.	Senior Staff Fellow	CB NEI
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Other: David S. Zee	M.D.	Visiting Scientist	CB NEI												
	M.D.	Senior Staff Fellow	CB NEI												
COOPERATING UNITS (if any) Department of Neurology, Johns Hopkins University															
LAB/BRANCH Laboratory of Sensorimotor Research															
SECTION Oculomotor Control Section															
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205															
TOTAL MANYEARS: 1.25		PROFESSIONAL: 1.0	OTHER: 0.25												
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<input type="checkbox"/> (a1) MINORS		<input type="checkbox"/> (a2) INTERVIEWS													
SUMMARY OF WORK (200 words or less - underline keywords)															
<p>Disease or trauma can weaken the extraocular muscles, making it difficult for patients to see clearly. The brain can compensate for these peripheral weaknesses, to some extent, by increasing the innervation sent to the muscles. We studied a patient's adaptation to a lateral rectus palsy when he habitually viewed with the weakened eye. As one expects based on previous publications, the vestibulo-ocular reflex, which moves the eye opposite to head movements, and the rapid, saccadic movements used to change visual fixation all showed adaptive changes. These systems operate in an "open-loop" manner, since they work too fast for visual feedback to have any influence over the individual eye movements. We showed that the pursuit system, which tracks smoothly moving visual targets, also exhibits adaptive increases in innervation. By studying this patient before and after corrective strabismus surgery we were also able to examine the contribution of adaptive mechanisms to the success of the surgical procedures.</p>															

Project Description:

Objectives: By studying human patients with peripheral nerve palsies, we hope to quantify the nature and range of the adaptive systems. Once the adaptive mechanisms are characterized, we hope to apply this understanding to study the central component of strabismus and the central response to extraocular muscle surgery. When the extraocular motor apparatus is weakened by disease or trauma, the brain attempts to compensate by increasing the innervation sent to the muscles. This can result in full or partial recovery, making adaptation an important clinical consideration. The characteristics and limits of adaptive mechanisms have been studied intensively over the last decade. Attention has been focused on the two rapid eye movement subsystems: the vestibulo-ocular reflex (VOR) and the saccadic eye movement system.

The VOR moves the eyes opposite to the perturbations of the head. It has a very short time delay (about 10 msec), and is said to operate as an "open-loop" system, since the vestibularly-induced eye movement is complete before visual feedback can affect it. The saccadic system moves the eye rapidly to change visual fixation. The duration of a saccade is less than 100 msec, and it too operates as an open-loop system.

It is not surprising, in the absence of visual feedback, that these two systems are under adaptive control. These long-term adaptive systems attempt to adjust the operation of the VOR and the saccade generators so that the movements are appropriate. When disease or trauma damage the peripheral motor apparatus, these adaptive systems compensate by altering the innervation within a few hours or days.

It is important to understand the function and limitations of these adaptive mechanisms, since they may mask symptoms in the early stages of disease, or may themselves become deficient. One of the key questions in the study of adaptive mechanisms relates to their generality. So far, all the systems known to be under adaptive control operate open-loop. We wondered whether closed-loop systems also exhibited adaptive properties.

We have been studying the smooth pursuit system in a patient with a lateral rectus palsy on one side, resulting in an inability to abduct one eye. By covering one eye at a time with a patch it is possible to force the patient to view the world through the eye that has inadequate muscle control. The pursuit system tracks smoothly moving objects with a very short latency (about 130 msec), and is usually considered to be a closed-loop control system. Nevertheless, we were able to demonstrate that the innervation sent to the eye muscles was increased adaptively when the patient habitually viewed with the weakened eye. Furthermore, the adaptation was specific for orbital position and movement direction.

The adaptive response compensates for the weakness of the paralyzed muscle. To measure this weakness, we assumed that the normal eye reflects the innervation sent by the brain, and hence the relationship between the position of the weak eye and that of the normal eye shows how the innervation must be changed. A Lancaster red-green test can be used to measure this relationship.

By making this test before and after strabismus surgery, it was possible to show that the brain always compensated for the direction and orbital-position dependent weakness.

Following strabismus surgery there was a spread of comitance over about a 10° field. This common clinical finding is being studied to see if it can be explained as the action of central or peripheral adaptive mechanisms.

Methods Employed: Since eye movements during smooth pursuit tracking are conjugate, the central innervation can be monitored by recording the movements of the normal eye, while it views, with the paretic eye covered. Accurate records are obtained with a wide bandwidth magnetic field/search coil technique. Human subjects are recorded by placing a coil, embedded in a silastic annulus, on the globe. The corneal bulge protrudes through the hole in the annulus, preventing slippage. With this technique very accurate recordings can be obtained, without touching the cornea.

Saccades and smooth pursuit movements are elicited by asking the patient to look at a small spot of light moved randomly by a computer. The coil technique limits the amount of time an experiment can run, hence a flexible computer program, called REX, was developed to allow us to specify the experimental protocol in advance, and have the entire experiment run automatically.

The muscle weakness is assessed with the Lancaster red-green test by plotting the static position of the weak eye against that of the normal eye. The offset of this curve reflects the patient's phoria, and the slope reflects the eye's mechanical gain at every point in the orbit.

The adaptive response is measured after the patient has been wearing a patch over the normal eye for nine days. The eye position records are digitally filtered to obtain velocity and acceleration traces. Analysis is then performed on the response in both the initial, or open-loop, and the final, or closed-loop, parts of the pursuit response.

Major Findings: As expected from previous work, the VOR and the saccadic system adaptively altered their innervation to compensate for the peripheral weakness when the patient habitually viewed with his paretic eye. In addition, we showed that this adaptation was quantitatively related to the specific weakness at every position in the orbit. In a new finding, the smooth pursuit system was shown to change adaptively by increasing its innervation for both the initial, open-loop period and the subsequent, closed-loop period of tracking. Adaptation occurred for tracking in both the on and off direction of the paretic muscle. High pursuit gains led to pendular oscillations. The adaptive changes of the vestibular, saccadic and pursuit movements depend on both orbital position and direction, and compensate for changes in orbital mechanics.

Prior to corrective strabismus surgery, the patient had an incomitant strabismus with a large esotropia. Immediately after surgery, the tropia was corrected, but not the incomitance. After several days of binocular viewing,

a range of comitance developed. When the patient's paretic eye was patched for several days, the incomitance returned. We infer from this that some adaptive mechanism is involved in the patient's recovery from surgery.

Significance to Biomedical Research and the Program of the Institute:

Study of the central adaptive mechanisms has a direct bearing on the ability to diagnose early diseases affecting the oculomotor system. The present study also attempts to determine to what extent central adaptive mechanisms are involved in strabismus disorders, and in the patient's recovery from strabismus surgery. Such studies may result in a new diagnostic test, perhaps with some ability to predict the effects of strabismus surgery.

Proposed Course: The basic research techniques we use to study adaptive mechanisms will be applied to patients with peripheral oculomotor palsies. The purpose will be to make a quantitative model of the smooth pursuit system, and to test its predictions against the effects of strabismus surgery.

NEI Research Program: Strabismus, Amblyopia, and Visual Processing--Ocular Motility and Strabismus (Disorders--Motor Neuro-Ophthalmic Disorders)

Publications:

Optican LM, Frank DE, Smith BM, Colburn TR: An amplitude and phase regulating magnetic field generator for an eye movement monitor. IEEE Trans on Biomed Eng BME-29:206-209, 1982.

Optican LM: Saccadic dysmetria, in Lennerstrand GL, Zee DS, Keller EL (eds): Functional Basis of Ocular Motility Disorders. Oxford, Pergamon (in press).

Zee DS, Butler PH, Optican LM, Tusa RJ, Gucer G: Effects of bilateral occipital lobectomies on eye movements in monkeys: Preliminary observations, in Roucoux A, Crommelinck M (eds): Physiological and Pathological Aspects of Eye Movements. The Hague, Junk Publishers (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00055-04 LSR
PERIOD COVERED October 1, 1981, to September 30, 1982			
TITLE OF PROJECT (80 characters or less) Visual and Oculomotor Functions of the Primate Superior Colliculus			
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT			
PI: Joanne E. Albano Ph.D. Staff Fellow LSR NEI Other: George F. Creswell B.S. Histologist LSR NEI			
COOPERATING UNITS (if any)			
LAB/BRANCH Laboratory of Sensorimotor Research			
SECTION Visuomotor Integration Section			
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205			
TOTAL MANYEARS: 1.5	PROFESSIONAL: 1.0	OTHER: 0.5	
CHECK APPROPRIATE BOX(ES)			
<input type="checkbox"/> (a) HUMAN SUBJECTS		<input type="checkbox"/> (b) HUMAN TISSUES	
		<input checked="" type="checkbox"/> (c) NEITHER	
<input type="checkbox"/> (a1) MINORS		<input type="checkbox"/> (a2) INTERVIEWS	
SUMMARY OF WORK (200 words or less - underline keywords) Pathways providing potential <u>sources of saccadic input</u> to the <u>primate superior colliculus</u> were investigated using <u>neuroanatomical tracing</u> techniques. Injections of horseradish peroxidase, a retrograde tracer, were made at various depths within the superior colliculus, including the stratum griseum intermediale, where electrical stimulation elicits saccadic eye movements with low thresholds. Other injections were made above and below this low threshold region. We found that numerous, diverse regions of the brainstem and frontal cortex project to the deep layers of the superior colliculus and subjacent mesencephalic reticular formation. However, sources of subcortical and frontal-cortical input to the intermediate layers of the colliculus are more limited than previously thought. These experiments indicate that three subcortical structures, the substantia nigra, the parabigeminal nucleus, and the rostral mesencephalic reticular formation, and a subregion of prearcuate cortex known as the frontal eye fields may provide the most prominent inputs to the presaccadic cells of the intermediate layers.			

Project Description:

Objectives: The purpose of these anatomical experiments was to determine the potential sources of saccade-related input to neurons in the intermediate layers of the superior colliculus. Our previous experiments (see references below) have shown that the superior colliculus participates in the selection or initiation of visually-guided saccades. The superior colliculus consists of three major divisions: the superficial layers, the intermediate layers, and the deep layers. Neurons in the superficial layers are activated via pathways from the retina and from visual cortical areas. Neurons in the intermediate layers discharge some 50-100 msec before the onset of a saccadic eye movement of an appropriate amplitude and direction. These saccade-related cells project to neurons in the paramedian pontine reticular formation and other brainstem regions that are believed to play a critical role in the generation of saccadic eye movements. Relatively little is known of the discharge characteristics of cells in the deep layers but our preliminary evidence indicates that in the monkey cells in the deep layers discharge in response to visual, auditory, and somesthetic stimuli. In last year's report, we described preliminary evidence that there are numerous sources of subcortical input to the superior colliculus but that the most prominent inputs arise from the substantia nigra, the parabigeminal nucleus and the mesencephalic reticular formation. During the past year we have extended these observations by quantifying differences in projection densities from the subcortical sites and by examining the topography of colliculus projection cells in the frontal eye fields.

Methods Employed: Eight rhesus monkeys were anesthetized and surgically prepared for neurophysiological recording in awake monkeys. After a recovery period, the superior colliculus was localized using microelectrodes to record visual and saccade-related activity and to elicit saccades by electrical stimulation. In order to identify cell bodies projecting to the colliculus, injections of horseradish peroxidase (HRP), a retrograde tracer, were made into the intermediate layers of the superior colliculus where electrical stimulation produced saccades with low threshold currents. Other injections were made dorsal or ventral to the low threshold region. After a survival time sufficient to allow retrograde transport of the enzyme, the animals were perfused, then the brains were sectioned and reacted for the presence of peroxidase using a modification of the Mesulam-TMB method to produce an optically-dense reaction product within the cell bodies. Using light microscopy, prepared sections were examined under light-field and dark-field illumination for cells labeled with the reaction product. The location of labeled cells were then plotted on projected drawings. Quantification of labeled-cell densities from each projection site were obtained by computing a Maximum Density Index (MDI) to assess the relative strengths of different sources of afferent input.

Major Findings: The distribution of HRP-labeled neurons depended upon the depth of the injection, that is, whether the injection site was centered on the superficial layers, intermediate layers or deep layers and subjacent mesencephalic reticular formation. Injections into the low-threshold, intermediate layer labeled neurons in a more restricted set of nuclei that are

implicated by previous studies in visual-oculomotor functions. By far the most prominent subcortical projections to the intermediate layers arose from the ipsilateral, anterolateral portion of the substantia nigra, the parabigeminal nucleus and the rostral mesencephalic reticular formation. Labeled cells in these sites were determined to have high MDIs. Other areas also contained labeled cells but with much lower densities; included in this group were the ipsilateral zona incerta, the ventral lateral geniculate, the sublentiform nucleus of the pretectal complex, the nucleus of the posterior commissure, and the parabrachial region of the mesencephalic reticular formation. MDIs indicated that projections from other sites were relatively weak; the paramedian pontine reticular formation, the contralateral prepositus hypoglossal nucleus, and the commissural projection from the contralateral superior colliculus are included in this group.

In subcortical sites, deep injections labeled neurons in more than 30 functionally-diverse nuclei including sources of potential visual, auditory, and somatosensory input. In addition to the projections already described, HRP-filled cells were observed in the ipsilateral hypothalamus, the inferior colliculus, locus ceruleus, as well as the contralateral facial, trigeminal, gracile, cuneate, the medial and interpositus nuclei of the cerebellum. MDIs suggest that the projections from zona incerta, the ventral lateral geniculate, the pretectum, the mesencephalic reticular formation, and the inferior colliculus are the most prominent inputs. An occasional labeled pretectal neuron is all that is seen in cases with injections of the superficial layers.

In frontal cortex, deep injections resulted in a widespread distribution of labeled neurons in cingulate, orbital, and dorsolateral cortex, indicating a functionally diverse input. On the other hand, intermediate layer injections resulted in a restricted area of labeled cells in prearcuate cortex in a region that has been called the frontal eye fields on the basis of previous electrical stimulation and lesion experiments.

These neuroanatomical experiments are in basic agreement with previous studies in the cat and monkey that show there are numerous diverse sources of afferents to the superior colliculus and underlying mesencephalic reticular formation. In addition, these experiments indicate that the sources of subcortical and frontal-cortical input to the intermediate layers of the superior colliculus are more limited than previously demonstrated. Subcortical afferents from the substantia nigra, the parabigeminal nucleus, and portions of the midbrain reticular formation and cortical input from the frontal eye-fields provide the major inputs to the saccade-related cells of the intermediate layers. The differences in the distribution of labeled cells that predominate after injections into the intermediate and deep layers suggest an organization of the primate superior colliculus that is consistent with the results of electrophysiological experiments. The intermediate layers contain cells that receive prominent projections from subcortical and frontal-cortical sites that are known to play an important role in visual-oculomotor functions and these cells discharge before saccades. These neuroanatomical experiments indicate that the deep layers receive afferent input from diverse sites that may provide multimodal input. Preliminary

electrophysiological experiments suggest that the cells in the deep colliculus layers reflect this multimodal input.

Significance to Biomedical Research and the Program of the Institute: An understanding of the organization of the neural pathways that give rise to preoculomotor signals in the brainstem is a prerequisite toward understanding oculomotor disorders that result from trauma or disease. Our earlier ablation experiments have shown that the superior colliculus plays an important role in the selection and initiation of visually-guided saccades. The present experiments shed light on the neuronal pathways that participate in these functions.

Proposed Course: The analysis of afferent inputs to the superior colliculus has thus far focused upon frontal-cortical areas lying rostral to the arcuate sulcus and subcortical areas from thalamus to medulla, including the basal ganglia and cerebellar nuclei. Future analysis will examine input from temporal and parietal cortex.

NEI Research Program: Strabismus, Amblyopia, and Visual Processing--Ocular Motility and Strabismus (Structure and Function--Conjugate Eye Movements)

Publications:

Albano JE, Wurtz RH: Deficits in eye position following ablation of monkey superior colliculus, pretectum, and posterior-medial thalamus. J Neurophysiol 48:318-337, 1982.

Albano JE, Mishkin M, Westbrook LE, Wurtz RH: Visuomotor deficits following ablation of monkey superior colliculus. J Neurophysiol 48:338-351, 1982.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00045-04 LSR
PERIOD COVERED October 1, 1981, to September 30, 1982			
TITLE OF PROJECT (80 characters or less) Visuomotor Properties of Neurons in the Thalamus			
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: David Lee Robinson Ph.D. Research Physiologist LSR NEI Other: Steven E. Petersen Ph.D. Staff Fellow LSR NEI			
COOPERATING UNITS (if any)			
LAB/BRANCH Laboratory of Sensorimotor Research			
SECTION Visuomotor Integration Section			
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205			
TOTAL MANYEARS: 3.0	PROFESSIONAL: 2.0	OTHER: 1.0	
CHECK APPROPRIATE BOX(ES)			
<input type="checkbox"/> (a) HUMAN SUBJECTS		<input type="checkbox"/> (b) HUMAN TISSUES	
		<input checked="" type="checkbox"/> (c) NEITHER	
<input type="checkbox"/> (a1) MINORS		<input type="checkbox"/> (a2) INTERVIEWS	
SUMMARY OF WORK (200 words or less - underline keywords) Four subdivisions of the <u>pulvinar</u> have been studied in awake monkeys to determine their contribution to <u>vision</u> and <u>visual behavior</u> . The beta portion of the <u>lateral pulvinar</u> contains a population of cells with <u>saccade-related activity</u> . Such cells discharge with eye movements, even when made spontaneously in the dark. Other cells are visual; most responses to stimulus movement are <u>pan-directional</u> (equal activity to all directions) while a few are <u>directionally-selective</u> (activity for some but not all directions). The mean latency is long and the standard deviation is large; these are also characteristic of its cortical target, area 7. The <u>inferior pulvinar</u> and adjacent alpha division of the lateral pulvinar are similar; both have a concentration of visual cells, with pan-directionality and directional selectivity common. The mean and standard deviation of latencies are different from the beta subdivision. The few cells with saccade-related activity are visually responsive and their eye movement activity is visually mediated. The <u>medial pulvinar</u> is a hybrid of the other pulvinar nuclei. There are functional differences between pulvinar subdivisions which correlate with their cortical target areas. 287			

Project Description:

Objectives: There are several routes over which visual information can reach the cerebral cortex. One pathway, from the retina to the lateral geniculate nucleus of the thalamus and then to striate cortex, is believed to be essential for the discriminative capacities of vision. A second major route goes from the retina to the superior colliculus and then to the pulvinar nuclei of the thalamus. The various nuclei of the pulvinar then have extensive connections with visual cortical areas. The function of such pathways outside the geniculostriate system is not well understood.

Anatomical studies have shown that the inferior pulvinar and the adjacent (alpha) part of the lateral pulvinar are strongly connected to striate cortex and the adjacent prestriate cortex. The posterior-dorsal (beta) part of the lateral pulvinar is strongly connected to inferior parietal cortex whereas the medial pulvinar is linked to the frontal eye fields and other cortical areas. The aim of the present set of experiments is to determine what functional properties in these cortical areas may be mediated by the afferents they receive from the subdivisions of the pulvinar.

Methods Employed: The discharge patterns of pulvinar neurons were studied while awake rhesus monkeys performed a variety of fixation and eye movement tasks. The animals learned to gaze at a spot of light (fixation point) projected on a tangent screen. They were reinforced with water for detecting the dimming of the spot of light. While the animals maintained fixation for brief periods of time, other lights were flashed onto or moved across the tangent screen to test the visual responses of pulvinar neurons. In order to study eye movements, the animals were trained to make a saccadic eye movement to fixate a new spot of light when the original fixation point was turned off. Eye movements were recorded with a surgically implanted scleral search coil. The following aspects of the experimental situation were controlled or monitored on-line with a digital computer: cell discharge patterns, eye movements, onset of light stimuli, and movement of various optical devices. Recordings were obtained from four subdivisions of the thalamus: inferior pulvinar, alpha subdivision of the lateral pulvinar, beta subdivision of the lateral pulvinar, and medial pulvinar. Small marks in the thalamus were made by passing current through the microelectrode at the sites of interesting cells. These lesions were later localized after perfusion and histological section of the brain, and such data were used to correlate physiological properties with anatomical locations.

Major Findings: There are several characteristics of cells in the beta subdivision of the lateral pulvinar which are similar to those of its cortical target, area 7 of posterior parietal cortex. Both areas have crude, if any, retinotopic organization. The cells in these regions have long mean latencies and large standard deviations. Their visual receptive fields are large, significantly larger than any in the geniculostriate system, and much larger than those in the other pulvinar subdivisions. There are no cells in these areas which require oriented stimuli to drive them, and many prefer large stimuli. In both of these structures there is a high incidence of cells with pan-directional responses to moving stimuli; they respond to stimulus movement

in all directions. These data suggest that the beta subdivision may act as a functional unit with area 7.

A second major population of cells found in the beta subdivision has not been demonstrated in area 7; these cells have saccade-related activity. The eye movement-related activity may be an excitatory response, an inhibitory pause, or a biphasic combination of inhibition followed by excitation. All of these types of activity can be seen with visually-guided saccadic eye movements or eye movements made spontaneously in total darkness. For the purely excitatory or inhibitory effects, this activity follows the saccadic eye movement. For cells with biphasic responses, the activity occurs during or slightly before the eye movement. This pattern of activity is characteristic of the beta subdivision of the lateral pulvinar.

The inferior pulvinar and the alpha subdivision of the lateral pulvinar have extensive interconnections with striate cortex (area 17) and the immediately adjacent cortical areas 18 and 19. The inferior pulvinar and alpha subdivision have many properties in common with each other. Both of these regions have similar proportions of cells responding to stimulus movement; the vast majority are pan-directional with only a minor subset of directionally-selective cells. All of the visual responses of these cells are organized relative to the retina; the location of the visual receptive field moves with the eye. The mean latency of visual response in the inferior pulvinar is 79 msec, 74 msec for the alpha subdivision; these are significantly shorter than for the beta subdivision of the lateral pulvinar. The standard deviations for these subdivisions are 16 and 15 msec, respectively, whereas the standard deviation is 45 msec for the beta subdivision.

The size of visual receptive fields increases with the distance from the fovea. The size of the visual receptive fields for the inferior pulvinar and alpha subdivision is significantly smaller than those for the other subdivisions of the pulvinar. The only characteristic in which these two subdivisions differ significantly is their preference for the size of visual stimuli. Most cells in the alpha subdivision respond equally to stimuli of all sizes, whereas about half of the neurons in the inferior pulvinar respond better to stimuli smaller than the excitatory receptive field. The cells in those areas which respond with eye movements have visual responses; their saccade-related activity disappears with eye movements made in the dark.

The medial pulvinar is connected with the frontal eye fields and the intermediate layers of the superior colliculus. The visual responses of cells here are equally distributed between pan-directional and directionally selective. The latency and standard deviation for the visual responses are short and narrow (comparable to the inferior and alpha subdivisions); the sizes of the visual receptive fields are large (comparable to the beta subdivision). There are many cells in the medial pulvinar with saccade-related activity; however, most have visual responses which can account for their apparent eye movement-related activity. There are a limited number of cells here which discharge with eye movements made spontaneously in total

darkness. These data suggest that many properties found in the medial pulvinar may be transmitted to the frontal eye fields.

Many cells in the visual system respond better to a visual stimulus which is the target for a saccadic eye movement than to the identical stimulus when fixation is maintained. This enhancement effect has been found in all of the subdivisions of the pulvinar tested and is present with comparable intensity and frequency in these regions.

Significance to Biomedical Research and the Program of the Institute:

Many regions of the brain receive visual information, and this has led to the hypothesis that different areas of the brain are specialized for different visual functions. For example, the striate and adjacent prestriate cortices are thought to be important for the fine, discriminative capacities of vision, posterior parietal cortex for visual attention, with the superior colliculus and frontal eye fields contributing to the visual initiation of eye movements. Experiments reported here attempt to determine the functional characteristics of the several subdivisions of the pulvinar nuclei. The finding that neurons in the beta subdivision of the lateral pulvinar have visual properties similar to those in area 7, with which it is strongly connected, suggests that this part of the thalamus provides the visual data to the cortical attentional system. The demonstration that neurons in the inferior pulvinar and alpha subdivision of the lateral pulvinar have strong visual responses suggests that these parts of the thalamus function in discriminative capacities of vision. Knowing which brain areas are related to different aspects of visual behavior and the mechanisms by which these functions are mediated will help in diagnosing the functional deficits in human patients with damage to the thalamus and cortex.

Proposed Course: Previous studies have demonstrated that a shift of attention will enhance the visual responses of cells in cortical area 7. Future studies will attempt to determine the spatial organization of the attentional mechanism which causes this enhancement. This will be accomplished by testing the visual responsiveness of area 7 neurons while the animal shifts its attention to different parts of the visual field. Similar experiments will be conducted on visual cells in the associated beta part of the lateral pulvinar to determine what role this part of the thalamus plays in mediating these attentional processes. A new set of experiments will evaluate the attentional and oculomotor capacities of humans with and without damage to posterior parietal cortex. Additional studies will investigate the capacities of individuals with damage to the temporal lobe to discriminate stimulus motion and motion after effects.

NEI Research Program: Strabismus, Amblyopia, and Visual Processing--Ocular Motility and Strabismus (Structure and Function--Conjugate Eye Movements)

Publications:

Wurtz RH, Goldberg ME, Robinson DL: Brain mechanisms of visual attention. Sci Am 246:124-135, 1982.

Keys W, Goldberg ME: Single neuron studies of attention, in Sheer D (ed): Attention: Theory, Brain Function, and Clinical Application. New York, Academic Press (in press).



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00102-03 LSR
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PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Role of Substantia Nigra in the Initiation of Eye Movements

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Robert H. Wurtz	Ph.D.	Chief	LSR	NEI
Other: Okihide Hikosaka	M.D., Ph.D.	Visiting Scientist	LSR	NEI

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Sensorimotor Research

SECTION

Visuomotor Integration Section

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 2.5	PROFESSIONAL: 1.5	OTHER: 1.0
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CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The basal ganglia of the brain are involved in the initiation of movement. A major output pathway of this structure is the substantia nigra pars reticulata in the brainstem. We have studied cells in this structure, and this report describes two types of neuronal responses that are related to fixation of gaze. Cells with the first type of response decreased their discharge rate following onset of a spot of light in the visual field, but only when the monkey was not looking at another spot of light. This suppression of the visual response was not due to the act of visual fixation but rather to the presence of the visual stimulus during fixation. The point in the visual field that gave the most vigorous suppression of the high background rate was always located near the fovea. The second type of response was to the offset of the spot of light on which the monkey was fixating; the presence of any spot of light, other than the fixation light, in the visual field reduced this response. Response of these two types of cells seems to occur only at the initiation or the termination of a series of visually-guided saccades and might signal the transition between spontaneous saccades and visually-guided saccades.

Project Description:

Objectives: The neural systems that control visual and oculomotor behavior extend throughout the brain. One part of this system, which we have identified over the past several years, is the substantia nigra pars reticulata. This structure in the midbrain represents an output pathway from the basal ganglia which has classically been regarded as related to the initiation of movement. We have studied cells in this structure because anatomical evidence indicated that this structure projects prominently to the superior colliculus which has been demonstrated, partly through our work over the last ten years, to be intimately related to the initiation of saccadic eye movements. In the two previous annual reports on this subject we have described the activity of cells in the substantia nigra that are related to visual processing and the initiation of saccadic eye movements. Our observations have indicated that the cells are related only to those eye movements made under certain contingencies: with saccades made to visual targets or saccades made to remembered targets. Spontaneous eye movements are not associated with cell discharges. In this report of work done in the last year, we will concentrate on a type of cell that is related to fixation of gaze, but is probably closely related to the mechanisms involved in the initiation of saccadic eye movement as well.

Methods Employed: We trained rhesus monkeys to fixate on a spot of light using procedures described in previous annual reports. In addition to our usual paradigm, in which the monkey looks at a spot of light and is rewarded for detecting the dimming of that spot of light, we trained the monkey to continue fixating even after the spot of light had gone off. The position of the eye was recorded using the magnetic search coil technique, eye movements were displayed and monitored by an on-line computer, and single-cell activity was displayed and monitored by the same computer.

Major Findings: The cells that we studied in the substantia nigra pars reticulata have a high frequency of discharge, 100 spikes per second being common. The response of these cells was always a decrease in this discharge rate. As we reported previously, a response to visual stimuli is one of the striking features of the substantia nigra cells. This visual response followed onset of the small spots of light in the visual field while the monkey looked at the fixation spot. As we were investigating these visual responses, we encountered a group of cells that decreased their discharge rate following onset, not of the spot of light in the visual field, but of the fixation spot. These cells, however, showed no response to other spots in the visual field while the fixation spot remained on. We, therefore, studied the characteristics of this type of cell in relation to visual fixation.

We have found that the decrease in discharge rate seen after the onset of the fixation spot is a type of visual response that is simply suppressed by the foveal stimulation associated with visual fixation. We established this point by having the monkey fixate on a spot of light which then went off; the monkey was required to maintain his fixation at that point of the visual field in the absence of the visual stimulus. During this continued fixation we flashed another spot of light and found that even though the monkey continued

to fixate there was a response to this second spot of light. Therefore, it was not the act of visual fixation that suppressed the response of these cells to light, but rather the fixation on the visual stimulus.

While the monkey was fixating in the absence of the visual stimulus, we could map the visual receptive field of the cells, and we found that these visual receptive fields were different from others we have studied: they were large, extending frequently by 40° both to the contralateral and ipsilateral visual fields, but the point in the field that gave the most vigorous suppression of the high background rate was always located near the fovea. The latency of the visual response had a mean of about 126 msec. Small spots of light were more effective than larger stimuli.

Although these cells can be regarded as having conventional visual responses to small spots of light, their unique feature is that this response is suppressed when another stimulus falls on the fovea when the monkey is fixating. In many respects these cells are similar to those observed in the inferotemporal cortex of monkeys and described in the annual report last year by Richmond and Wurtz.

A second type of response related to the monkey's fixation of gaze was the response to the offset of the spot of light rather than the onset. In order for this response to be observed the monkey had to be fixating on the spot of light and no other spot of light could be present in the visual field.

One of the important points in considering the significance of these cells is that these fixation contingent responses occur at only two times: with the appearance of a spot of light that the monkey is going to fixate; with the disappearance of a spot of light that the monkey has been fixating. The cells do not respond when a spot of light that the monkey is fixating simply moves from one part of the visual field to another and the monkey follows the spot of light by making a series of saccades. Response of the cells, therefore, seems to signal the initiation or the termination of a series of visually-guided saccades but not the continuation of such behavior. We think, therefore, that these cells might facilitate the onset and termination of a series of visually-guided saccades. This is consistent with our observation that other cells in the substantia nigra are related to the initiation of saccades and to visually-guided behavior. Since the cells project directly to the superior colliculus, it is quite possible that these cells act through the superior colliculus on the brainstem oculomotor areas.

Significance to Biomedical Research and the Program of the Institute: Our goal in these research projects is not just to study cells in the monkey brain but to try to understand the neural circuits that underlie the initiation of visual and oculomotor behavior. The experiments described in this report, along with those of the preceding two reports, indicate the type of activity in an output pathway from a major motor system within the brain, the basal ganglia. The basal ganglia are suspected to be involved in several diseases including Parkinson's disease and Huntington's disease. There are indications that both of these diseases involve abnormalities of oculomotor control. Our hope is that an understanding of these cells, and eventually the circuitry in

Project No. Z01 EX 00102-03 LSR

the brain related to oculomotor behavior, will aid in the evaluation of deficits in these patients with diseases of the basal ganglia.

Proposed Course: Our experiments will concentrate on the relation of the substantia nigra pars reticulata to the superior colliculus and on the effect of damage of these areas on the initiation of saccadic eye movements and visual fixation.

NEI Research Program: Strabismus, Amblyopia, and Visual Processing--Ocular Motility and Strabismus (Structure and Function--Conjugate Eye Movements)

Publications:

Wurtz RH: Neural mechanisms in the substantia nigra for the initiation of saccadic eye movements. Freiburger Universitatsblatter 74:103-105, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00109-02 LSR
PERIOD COVERED October 1, 1981, to September 30, 1982			
TITLE OF PROJECT (80 characters or less) Visual Motion Processing in the Primate Brain			
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT			
PI: Robert H. Wurtz	Ph.D.	Chief	LSR NEI
Other: William T. Newsome	Ph.D.	Staff Fellow	LSR NEI
	Akichika Mikami	M.D., Ph.D.	Visiting Scientist LSR NEI
COOPERATING UNITS (if any)			
LAB/BRANCH Laboratory of Sensorimotor Research			
SECTION Visuomotor Integration Section			
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205			
TOTAL MANYEARS: 3.0	PROFESSIONAL: 2.0	OTHER: 1.0	
CHECK APPROPRIATE BOX(ES)			
<input type="checkbox"/> (a) HUMAN SUBJECTS		<input type="checkbox"/> (b) HUMAN TISSUES	
		<input checked="" type="checkbox"/> (c) NEITHER	
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS			
<p>SUMMARY OF WORK (200 words or less - underline keywords)</p> <p>Processing of visual information continues beyond the striate cortex in the extrastriate visual areas of the primate cerebral cortex. One of these areas, the middle temporal area, has many neurons that respond selectively to the direction of motion of visual stimuli. We have studied two aspects of cells in this area in awake monkeys able to move their eyes and respond to visual stimuli. The first concerns the way in which these cells respond to the apparent motion of a series of flashed spots of lights that do not move but appear to a human observer to have moved. MT cells show the same directionally selective response to such flashed stimuli as they do to moving stimuli if the frequency of flashes is high enough. This frequency falls into the same range as that producing apparent motion for human observers. The second investigation has shown that areas adjacent to MT have cells that discharge in relation to pursuit eye movements. Histological analysis of myelin stained sections of this region reveals distinct anatomical areas. These experiments indicate that there are a group of prestriate areas probably related to analysis of motion which are probably utilized for both visual perception and oculomotor control.</p>			
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Project Description:

Objectives: Experiments in anesthetized animals have demonstrated the existence of several visual areas in the occipital lobe of monkeys which can be distinguished on the basis of cell structure, anatomical connections, visual topography and neuronal response properties. We have investigated one of these areas, the middle temporal visual area (MT), employing single-cell recording techniques in awake, behaving monkeys. In the past year we have focused our efforts on two specific investigations of MT neurons. The first has dealt with the phenomenon of apparent motion. In psychophysical experiments, human observers can perceive motion when viewing an appropriate sequence of flashed stimuli even though each individual stimulus is in fact stationary (apparent motion). MT contains a high proportion of direction selective (DS) neurons and is thus thought to play a role in the processing of motion information. We have, therefore, begun an investigation of the effects of sequentially flashed stimuli on MT neurons with the goal of defining the parameters of such sequences that are necessary to elicit DS responses. The second specific investigation has been a study of neurons in an area adjacent to MT which combines direction selective visual responses with directional discharges related to smooth pursuit eye movements. This area of cortex receives a direct projection from MT and probably represents a higher level of processing of information generated in MT.

Methods Employed: Behavioral control, stimulus presentation, data acquisition, and on-line analysis of monkey behavior and neuronal response patterns was done by a digital computer. Rhesus monkeys were trained on four tasks: fixate a spot of light and ignore other stimuli; make a saccade from the fixation point to a peripheral stimulus; track a moving stimulus with smooth pursuit eye movements; make a saccade from the fixation point to a moving peripheral stimulus and pursue the stimulus with smooth pursuit eye movements. Eye movements were monitored by the magnetic search coil technique. Electrolytic marking lesions were made at selected recording sites for subsequent histological identification of recording locations. In several monkeys the Gallyas silver stain for myelinated fibers was used to clearly delineate anatomical areas.

Major Findings: Experiments on apparent motion were performed while the monkey fixated on a small, stationary spot of light. Direction and velocity tuning for each MT neuron were determined using a computer controlled series of smoothly moving stimuli. Sequentially flashed stimuli (0.7° square) then stepped through the neuron's receptive field in the preferred direction and in the null direction. The stroboscope flash duration was 10 usec.

In general, low flash frequencies (interflash interval, IFI = 150 msec) elicited equivalent responses to sequences in both preferred and null directions: the discharge for each direction was a series of transient responses, one to each stroboscopic flash. However, as the flash frequency was increased (at constant mirror velocity), a transition point was reached where neuronal discharge ceased for the null direction and the neuron became directionally selective. For a mirror velocity of $15^{\circ}/sec$, this critical frequency ranged from 12 Hz (IFI = 83 msec, interflash distance, IFD, = 1.25°)

to 18 Hz (IFI = 56 msec, IFD = .84⁰) for the neurons we studied. As mirror velocity was increased, a higher flash frequency was required to elicit directional selective responses. In other experiments the IFI and IFD were varied independently of each other. At appropriate values of IFD, directionally selective responses could generally be elicited for IFI's up to 70 msec, and occasionally up to 200 msec. Various optimal values of the IFI for the perception of apparent motion have been reported in the psychophysical literature: 20-30 msec (Sperling, 1979), up to 70 msec (Morgan and Turnbull, 1979), up to 40 msec (Morgan, 1980). Values of IFI which are optimal for the perception of apparent motion in humans seem to be a subset of those which will elicit DS responses in MT neurons in monkeys. Continued experiments of this nature should allow a precise definition of the relation between perception of apparent motion and "motion-like" neuronal responses in MT.

The second specific investigation concentrated on an area adjacent to MT. As we reported last year, neurons in this area have conventional large receptive fields, but in addition, discharge when the monkey makes smooth eye movements in a particular direction. We have now found that the pursuit related discharge continues in total darkness so it is clearly generated by the motor task and not by spurious visual stimulation during the task. However, this response cannot be considered "motor" in the sense of being responsible for the initiation of pursuit eye movements since the discharge begins after the eye movement begins. While we have no precise teleological explanation for these responses, it seems likely that such neurons would be useful during the maintenance of pursuit eye movements, or in dealing with the perceptual consequences of those eye movements.

In the course of our studies we have identified two zones in the superior temporal sulcus which can be identified histologically using myelin stains. Both of these contain neurons related to pursuit eye movements. The first is a very heavily myelinated zone on the anterior bank of the sulcus occupying a position roughly similar to that of MT on the posterior bank. This zone stands out clearly in well stained sections and has averages 20 mm² in the two hemispheres studied to date. The second zone occupies cortex between MT and the anterior bank area and includes the fundus of the sulcus. This zone tends to be more lightly myelinated but is primarily distinguished by the orientation of its fibers and by the more pronounced appearance of the bands of Baillarger. Both of these zones appear to be subdivisions of area PG of von Bonin and Bailey. Identification of these zones anatomically allows us to relate neuronal activity to specific anatomical structures. These newly identified zones along with MT constitute a region in the STS that has in common the processing of information concerning the direction of motion and a dense myeloarchitecture relative to surrounding cortex.

Significance to Biomedical Research and the Program of the Institute: An understanding of the basic mechanisms of visual function is a prerequisite for the diagnosis and treatment of pathologies of central visual structures. Issues of visual information processing and visuomotor integration can be addressed most directly by an analysis in awake behaving animals. We are applying these approaches to visual function in a higher-order extrastriate visual area. We hope that these studies will constitute a significant step in

Project No. Z01 EY 00109-02 LSR

the attempt to understand the intricate stages of normal visual processing in monkey and, by inference, in man.

Proposed Course: Work in the coming year will concentrate on cell activity in MT and adjacent areas related to both oculomotor control of pursuit eye movements and to perception of moving stimuli.

NEI Research Program: Strabismus, Amblyopia, and Visual Processing--Ocular Motility and Strabismus (Structure and Function)

Publications: None

Laboratory of Vision Research

ANNUAL REPORT
NATIONAL EYE INSTITUTE
October 1, 1981 - September 30, 1982

REPORT OF THE CHIEF, LABORATORY OF VISION RESEARCH
Gerald J. Chader, Ph.D.

This year has been one of great change for the LVR, a time of both rearrangement and consolidation. Under the leadership of Dr. Jin Kinoshita over the last decade, the Laboratory developed into perhaps the foremost vision research grouping in the world. The programs of the lab grew along several independent lines during this period, with projects on lens, retina and cornea in diverse areas of biochemistry, cell biology, physiology, embryology and pathology. The main thrust of, and unifying thread through each of these projects has been an attempt to understand better the normal functioning of eye tissues and to relate these basic findings to diseases of the ocular system.

To achieve these goals and to recognize the development of several key projects and personnel, the LVR has now been reorganized into seven sections.

1. Section on Experimental Pathology: Dr. Kuwabara continues to be a leading force in studies relating ocular structure, function and pathology. His laboratory has been a primary international training center in this area both in the theoretical and diagnostic aspects of ocular pathology and in technical aspects of actual problem solving. Most recently, Dr. Kuwabara has developed a primate model for the disease gyrate atrophy. This is a disease in which a hereditary enzyme defect (i.e. decreased ornithine aminotransferase activity) and high serum ornithine level leads to retinal degeneration and blindness. In Dr. Kuwabara's experimental model, the effects seen are quite specific and parallel the course of the disease in humans, making this an extremely important advance in the study of inherited retinal degenerations.
2. Section on Experimental Anatomy: This is a new section in the LVR, headed by Dr. W. G. Robison, Jr. that is mainly devoted to better understanding the dynamic exchange between the photoreceptor cells of the neural retina and the pigment epithelium (PE). Dr. Robison has made great advances in our understanding of both the development of these tissues and many of the deleterious changes which occur during aging. In particular, he has elucidated the delicate balance between vitamin A and vitamin E in maintaining homeostasis and normal functioning in the photoreceptor-PE functional unit.
3. Section on Cell Biology: This also is a new section, recognizing Dr. Paul O'Brien's contributions in the area of retinal biochemistry. Most recently, Dr. O'Brien has focused on the question of the mechanism of disc shedding from photoreceptor outer segments and phagocytosis of these packets by PE cells. He has shown that the process is linked to the general circadian rhythms of the body as controlled by the normal light/dark cycle. Dr. O'Brien also has found that the rate of outer segment renewal in a canine model for retinal degeneration is abnormally slow, making this a new and provocative

lead to follow in the study of the etiology of human retinal degeneration.

In Dr. O'Brien's section, Dr. Barbara Battelle has also made excellent progress in understanding the role of neurotransmitters in the retina. In particular, she has identified the specific neurotransmitter released from efferent fibers in the retina in the visual system of *Limulus polyphemus*. These fibers innervate the retina from the CNS and could modulate photoreceptor sensitivity.

4. Section on Retinal Metabolism: With the formation of Dr. O'Brien's section, the efforts of the remaining members of this section focused on two major areas. First, they investigated the role of cyclic nucleotides in the visual process. In this area, several new neurotransmitter/ neuromodulator receptors were identified in retina and PE. It was especially surprising to find the PE reacting to substances such as glucagon, VIP, and β -adrenergic agents, indicating a definite role for these messengers in regulating PE cell function. The second area of interest, led by Dr. Barbara Wiggert, has focused on the potential role of vitamin A binding proteins as transport vehicles for retinoids within and between tissues. In particular, a specific protein has been identified in the subretinal space (the Interphotoreceptor Retinol-Binding Protein) that has most of the characteristics one would expect (e.g. differences in light/dark binding) of a protein involved in vectorial movement of vitamin A between retina and PE.
5. Section on Lens and Cataract: Under the general guidance of Dr. Kinoshita, this unit remains the single most productive group of investigators in the lens field. Dr. Samuel Zigler has developed, for example, an excellent model for studying cataractous changes in the lens induced by photosensitizers and oxidative conditions. Dr. Paul Russell has continued to work on lens membrane changes in the early stages of cataract development. He has pinpointed important changes in membrane constituents and in enzyme inactivation which could lead to cataract formation. Dr. Peter Kador has made important advances in the pharmacology of the enzyme aldose reductase. These findings have broad implications both for the formation of cataracts and for the general area of diabetes research.
6. Section on Experimental Immunology: This section has been formed in recognition of the importance and growth of this area under the leadership of Dr. Igal Gery. Dr. Gery has developed programs in both basic immunology (e.g. macrophage involvement in ocular function) and in immunopathology (e.g. models for autoimmune uveitis). He has also collaborated with several other members of the LVR and of the NEI Clinical Branch. Particularly exciting is the ongoing work on the S-antigen and its role in ocular uveitis.
7. Section on Experimental Biology: Dr. A. Coulombre headed this section for many years and was notably successful in coordinating its diverse research areas, supplying ideas, and interacting with individual investigators. It was a great scientific loss and a truly sad personal loss to all of us for "Chris" to have retired this year. His scientific contributions have been aptly summarized in the July 1982

issue of Developmental Biology. His personal attributes of integrity, industry, selflessness and enthusiasm are more difficult to document and commit to paper, but are no less real and of value to the people he has guided and worked with throughout his career.

Also in this section, Dr. Donald Puro has established himself as a leader in the emerging field of the neurobiology of the retina, applying sophisticated techniques of tissue culture and electrophysiology to help elucidate basic questions of retinal cell interactions and development. It has become clear from his work that extraocular signals (e.g. glucocorticoid hormones) play critical roles in early retinal development, a finding that, as in the work of Dr. O'Brien, demonstrates that the retina is heavily influenced by extraocular factors. In a somewhat similar vein, Drs. Ralph Nelson and Andrew Mariani have used electrophysiological techniques to advantage in their study of the morphology and interactions of inner retinal neurons. This study has not only led to a method for discriminating between different subpopulations of the major types of retinal neurons but a better understanding of signal processing between the photoreceptor cell and the brain.

In summary, this last year has been a time of significant changes, loss of key personnel, and reallocation of resources for the LVR. The laboratory has, however, maintained a high standard of productivity, as can be seen in the following individual project reports.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00066-05 LVR

PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Neurotransmitter Chemistry of Retinal Neurons

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Barbara-Anne Battelle	Ph.D.	Senior Staff Fellow	LVR	NEI
Other:	Judith A. Evans	Ph.D.	Staff Fellow	LVR	NEI
	Donald G. Puro	M.D.	Medical Officer	LVR	NEI

COOPERATING UNITS (if any)

State University of New York, Stony Brook, NY

Institute for Sensory Research, Syracuse University, Syracuse, NY

Marine Biological Laboratory, Woods, Hole, MA

LAB/BRANCH

Laboratory of Vision Research

SECTION

Section on Cell Biology

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

2

PROFESSIONAL:

2

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A combined biochemical and anatomical study is underway to identify chemical neurotransmitters in retinal neurons and in neurons of the visual pathway, to examine the development of neurotransmitter systems in retina, and to determine the role of chemical neurotransmitters in the processing of visual information. Two systems are being investigated: (1) the relatively simple visual system of Limulus polyphemus and (2) developing mammalian retinal neurons in intact retinas and in monolayer cell culture. Work with the simple visual system has led to the identification of the biogenic amine octopamine as a neurotransmitter released from retinal efferent fibers; i.e. fibers which originate from cells in the brain and innervate the retina. Our studies suggest that this amine may alter visual sensitivity and may be important in maintaining normal photoreceptor cell function. Studies of the intact developing retina have shown that at least two neurotransmitters, GABA and acetylcholine appear very early in normal retinal development. The regulation of development of these two neurotransmitter systems is being investigated using monolayer cell cultures.

Project Description:

Objectives: Superimposed upon the anatomical wiring diagram of neurons in visual systems is a complex synaptic chemistry. Knowledge of the synaptic chemistry of these neurons is critical to our understanding of visual processing in normal retinas as well as the causes of blindness. Our aim is to (1) identify neurotransmitter molecules in the visual system (2) learn how the development of neurotransmitter systems is controlled in retinas and (3) understand the function of individual neurotransmitters in the processing of visual information.

Methods Employed: High voltage paper electrophoresis and modern liquid chromatographic techniques are employed to study the synthesis, storage and metabolism of putative neurotransmitters. Sites of synthesis, uptake and storage of putative neurotransmitters are localized using light and electron microscopic autoradiography, histofluorescence and immunocytochemistry. Monolayer cell cultures of retinal neurons grown in serum and serum-free conditions are being used to study the control of the development of neurotransmitter systems.

Major Findings: Our major findings are summarized below.

- I. First identification of a neurotransmitter of retinal efferent fibers.
 - a. The biogenic amine octopamine is synthesized and stored in fibers which project from the brain to the eyes of the horseshoe crab Limulus polyphemus.
 - b. Newly synthesized octopamine is released from these efferent fibers with depolarization and the release exhibits all of the characteristics of classical neurotransmitter release.
 - c. Anatomical studies revealed that octopamine-containing efferent fibers innervate the photosensitive membrane of ventral photoreceptor cells exclusively.
 - d. Octopamine induces large increases in the level of cAMP in both Limulus lateral eyes and in ventral photoreceptor cells.
 - e. Preliminary studies suggest that octopamine can induce changes in the level of phosphorylation of photoreceptor cell proteins.

Our studies indicate that octopamine is very likely a neurotransmitter released from retinal efferent fibers in the Limulus visual system, and that this amine can cause profound changes in the biochemistry and function of target cells in the eyes including the photoreceptor cells themselves.

- II. Early development of neurotransmitter systems in intact rat retinas.
 - a. The synthesis of two neurotransmitters acetylcholine and GABA is detected very early in the development of the retina, approximately two weeks before synapses are seen in the retina with the electron microscope.
 - b. The development of GABA and acetylcholine synthesis and storage follows a very similar time course suggesting that these two neurotransmitter systems mature at similar rates.
 - c. The rates of GABA and acetylcholine synthesis and the activity of choline acetyltransferase, the enzyme which controls acetylcholine synthesis, increase dramatically in retina when structural synapses begin to form.
 - d. An examination of the effect of light deprivation on the activity of choline acetyltransferase prepared from developing rat retinas revealed no change. This result indicates that light is not required for the normal maturation of some neurotransmitter functions.

III. Studies of the development of neurotransmitter systems in monolayer cultures of retinal neurons.

a. Dissociated retinal neurons from embryonic rats grown in monolayer culture synthesize both acetylcholine and GABA.

b. GABA uptake into neurons is also observed in these young cultures of embryonic retina cells.

c. To date our emphasis has been on studying factors which may influence cholinergic development in the retinal cultures. The availability of extracellular choline is a critical factor determining rates of acetylcholine synthesis. Hormones and other factors which are known to influence the release of acetylcholine from cultured retinal neurons have little or no influence on acetylcholine synthesis.

Significance to Biomedical Research and the Program of the Institute:

Efferent innervation to retinas is common among many animals. The function of this efferent innervation is only beginning to be understood. There is now evidence from several different species that efferent innervation controls circadian changes in the sensitivity of the eye to light, photoreceptor cell sensitivity and photoreceptor cell membrane turnover. Our experiments have suggested that efferent innervation can have profound effects on the biochemistry of photoreceptor cells. Therefore, the possibility should be explored that some retinal disease may be caused by a defect in efferent innervation. Our identification of neurotransmitter in retinal efferent fibers of the Limulus visual system, a preparation that has been central to our understanding of basic visual progresses, will allow for detailed investigations of the biochemical and electrophysiological mechanisms underlying efferent control of visual function.

Normal processing of visual information by the adult retina requires establishment of appropriate synaptic connections among retinal neurons during development. Studies of neurotransmitter systems in the normal developing mammalian retina, in addition to revealing fundamental features of neuronal differentiation in a healthy tissue, lay the necessary ground work for future studies of possible environmental and genetic influences on retinal development.

Proposed Course: Studies of the effect of octopamine on the biochemistry and function of photoreceptor cells will be pursued. Emphasis will be placed on characterizing the changes in phosphorylation of photoreceptor proteins induced by light and by octopamine. Our studies of the development of neurotransmitters in mammalian retinas will focus on a study of factors which influence the synthesis and release of acetylcholine in retinal cultures. Our studies then will be extended to the GABA system.

NEI Research Program: Retinal and Choroidal Diseases--Retinal Organization, Neurotransmission, and Adaptation

Publications:

Puro DG, Battelle BA, Hansmann RE: Development of cholinergic neurons in rat retina. Devel Biol 91:138-148, 1982.

Kaupp UB, Malbon CC, Battelle BA, Brown JE: Octopamine stimulated rise in cAMP in Limulus ventral photoreceptors. Vision Res (in press).

Battelle BA, Evans JA, Chamberlain SC: Efferent fibers to Limulus eyes synthesize and release octopamine. Science 216:1250-1252, 1982.

Battelle BA, Puro DG, Hansmann KH: Early development of cholinergic function in rat retina. Society for Neuroscience Abstracts 7:399, 1981.

Evans JA, Battelle BA: EM autoradiographic localization of octopamine to efferent fibers in Limulus ventral eye. Society for Neuroscience Abstracts 7:278, 1981.

Evans JA, Battelle BA: Efferent fibers to Limulus eyes synthesize, store and release octopamine. Invest Ophthalmol Vis Sci 22:(suppl)284, 1982.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00015-17 LVR

PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

The Cell Biology of the Vertebrate Retina

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Paul J. O'Brien	Ph.D.	Research Chemist	LVR	NEI
Other:	James P. Alligood	B.S.	Biologist	LVR	NEI
	Nancy Philp	Ph.D.	Post-Doctoral Fellow	LVR	NEI
	Peter A. Dudley	Ph.D.	Staff-Fellow	LVR	NEI

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Vision Research

SECTION

Section on Cell Biology

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

1.6

PROFESSIONAL:

1.4

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

 (a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER (a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Opsin labeling with galactose was found to occur at a constant rate throughout the diurnal cycle. Thus, neither synthesis nor intracellular transport of opsin appears to exhibit any circadian rhythm.

Iodination of bovine pigment epithelium followed by SDS gel electrophoresis revealed a reproducible pattern of plasma membrane proteins, several of which were also labeled on incubation of eyecups with radioactive glucosamine. Thus these are glycoproteins which may mediate recognition of outer segment membranes in preparation for phagocytosis.

Project Description:

Objectives: Many interactions between macromolecules and cell membranes are mediated by the sugar molecules bound to one of the interacting surfaces. This project was designed to determine where and when sugars are added to rhodopsin and what role they play in the transport and assembly of rhodopsin into disc membranes and in the process of shedding and phagocytosis of disc membranes. In addition, biochemical correlates to circadian photoreceptor shedding will be sought, particularly in relation to glycoprotein synthesis and function. Finally specific carbohydrate receptors will be sought on pigment epithelial microvilli which could mediate interactions with photoreceptors.

Methods Employed: Ordinary biochemical techniques were used, such as incubation of retinas and pigment epithelium with radioactive precursors, cell fractionation, SDS gel electrophoresis, scintillation and gamma counting, and autoradiography.

Major Findings: Radioactive galactose was incorporated into opsin in rat retinas incubated *in vitro* at various times throughout a normal 12-hour light and 12-hour dark cycle. The rate of labeling was nearly constant at all times.

Iodination of bovine and rat eye cups gave reproducible and similar patterns of labeled pigment epithelium (PE) plasma membrane proteins after detergent extraction and gel electrophoresis. Several of the major iodinated species could also be labeled by incubation of the eyecups with radioactive glucosamine, thus identifying the surface proteins as glycoproteins.

Significance to Biomedical Research and the Program of the Institute: Circadian photoreceptor shedding requires at least two hours of darkness and approximately a 24-hour interval between shedding events presumably representing biosynthetic reactions. Previous work showed that rhodopsin polypeptide synthesis and core glycosylation occur at a constant rate. Galactose addition, which has been implicated in *cis*-to *trans*-Golgi translocations, likewise seems fairly constant. Thus, these steps in synthesis and intracellular transport may be part of the 24-hour interval but not part of the 2 hours of dark reactions needed to sustain normal shedding.

The shed photoreceptor membranes are phagocytized by the PE by a mechanism that may involve specific recognition by receptors on the PE cell surface. Characterization of these receptors permits comparison of the PE cell surface components of normal retinas with those affected with inherited degenerative disorders. Likewise the identification of mechanisms involved in photoreceptor shedding provides an opportunity to examine potential defects in dystrophic retinas.

Proposed Course: A search will be made for modifications to proteins and phospholipids, such as methylation reactions, that occur during the two hour period of darkness required for shedding. In addition other PE cell surface components such as proteoglycans will be sought.

NEI Research Program: Retinal and Choroidal Diseases--Photoreceptors, Visual Pigments, and Phototransduction.

Publications:

O'Brien PJ: Purification of rhodopsin on agarose, in Packer L (ed): Methods in Enzymology, Vol 81, Biomembranes, Part H, Visual Pigments and Purple Membranes, I. New York, Academic Press, 1982, pp 141-144.

O'Brien PJ: Glycosylation of rhodopsin, in Packer L (ed): Methods in Enzymology, Vol 81, Biomembranes, Part H, Visual Pigments and Purple Membranes, I. New York, Academic Press, 1982, pp 783-788.

Philp NJ, O'Brien PJ: Cell surface membrane proteins of the retinal pigmented epithelium. Invest Ophthalmol Vis Sci 22(suppl):228, 1982.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00016-15 LVR

PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

The Biochemistry of Normal and Dystrophic Retinas

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Paul J. O'Brien	Ph.D.	Research Chemist	LVR	NEI
Other:	James P. Alligood	B.S.	Biologist	LVR	NEI
	Nancy Philp	Ph.D.	Post-Doctoral Fellow	LVR	NEI

COOPERATING UNITS (if any)

School of Veterinary Medicine, University of Pennsylvania

LAB/BRANCH

Laboratory of Vision Research

SECTION

Section on Cell Biology

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.9	0.7	0.2

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Opsin synthesis was measured in miniature poodles affected with progressive rod-cone degeneration. Photoreceptors develop normally but begin to degenerate after the dog is fully grown. At all ages studied the rate of rod outer segment renewal was about half the normal value. Opsin synthesis, however, occurred at the normal rate until advanced stages of the disease when photoreceptor cell death was apparent. Thus the defect may involve photoreceptor membrane assembly rather than synthesis.

Project Description:

Objectives: The renewal of photoreceptor cell outer segments is a continuous process which is impaired in some pathological conditions such as progressive degeneration or developmental anomalies of the retina. The purpose of this project is to examine biochemical events unique to the retina, especially the synthesis of photoreceptor membrane and pigment epithelium (PE) cell surface components, in the retinas of vertebrates which can be affected by inherited retinal degenerations.

Methods Employed: Ordinary biochemical techniques were used, such as incubation of retinas and pigment epithelium, cell fractionation, isolation of rod outer segments by density gradient centrifugation, and SDS gel electrophoresis.

Major Findings: The synthesis of membrane proteins, particularly opsin, was found to occur at an identical rate in 14-month-old normal miniature poodles and in littermates affected with progressive rod-cone degeneration, an inherited disease. The rate of rod outer segment renewal, revealed by autoradiography following intravitreal injection of labeled leucine, was half the normal value. Only when photoreceptor cell death was apparent did the synthesis of opsin begin to decrease.

Iodination of normal Irish setter pigment epithelium produced a pattern of labeled proteins on SDS gels similar to that seen with bovine and rat PE.

Significance to Biomedical Research and the Program of the Institute: The reduced rate of rod outer segment renewal in the affected miniature poodle is not the result of reduced membrane synthesis since opsin synthesis occurs at a normal rate. Thus, it is possible that the assembly of membranes may be defective. This represents a new class of degenerative disorders which closely mimics retinitis pigmentosa in humans.

The iodination procedure will make possible a comparison of PE surface receptor proteins in normal and affected animal models of retinal degeneration.

Proposed Course: Both Irish setters and miniature poodles with inherited retinal degenerations will be studied further to search for specific biochemical defects in the assembly of photoreceptor membrane components. Both leucine and fucose incorporation will be studied to assess rod and cone metabolic processes. Plasma membrane proteins will be studied in pigment epithelium from normal and RCS rats as well as normal and affected dogs in search of a missing or defective receptor protein involved in recognition and phagocytosis of rod outer segments.

NEI Research Program: Retinal and Choroidal Diseases--Developmental and Hereditary Disorders

Publications:

Aguirre G, Alligood J, O'Brien P, Buyukmihci N: Pathogenesis of progressive rod-cone degeneration in miniature poodles. Invest Ophthalmol Vis Sci (in press).

Aguirre G, Farber D, Lolley R, O'Brien P, Alligood J, Fletcher RT, Chader G: Retinal degenerations in the dog: III Abnormal cyclic nucleotide metabolism in rod-cone dysplasia. Exp Eye Res (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00149-09 LVR

PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Ultrastructure and Function of the Pigment Cells of the Eye

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. Gerald Robison, Jr.	Ph.D.	Chief, Section on Experimental Anatomy	LVR	NEI
Other:	Toichiro Kuwabara	M.D.	Chief, Section on Experimental Pathology	LVR	NEI
	Martin L. Katz	Ph.D.	Staff Fellow	LVR	NEI
	Roland K. Herrmann	M.D.	Visiting Fellow	LVR	NEI
	John G. Bieri	Ph.D.	Chief, Section on Nutritional Biochemistry	LNE	NIADDK

COOPERATING UNITS (if any)

Laboratory of Nutrition and Endocrinology, NIADDK

LAB/BRANCH

Laboratory of Vision Research

SECTION

Section on Experimental Anatomy

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

2.1

PROFESSIONAL:

1.2

OTHER:

0.9

CHECK APPROPRIATE BOX(ES)

 (a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER (a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Intracellular accumulations of lipofuscin (aging pigment) occur gradually with age and can be accelerated by vitamin E deficiency as a model for this aspect of aging. Supposedly, lipofuscin originates from highly oxidized cell products which polymerize and form insoluble, autofluorescent granules within the cytoplasm. Our studies confirm the classical concept that membrane lipids containing polyunsaturated fatty acids (PUFA) contribute significantly to lipofuscin formation in the retinal pigment epithelium (RPE). However, dietary levels of vitamin A appear to be even more important in determining the rate of lipofuscin accumulation. Even a RPE with no source of ingestable rod outer segment membranes which contain PUFA will accumulate large amounts of lipofuscin in a relatively short time if the level of vitamin A is high, but will accumulate almost none if vitamin A is absent. In fact, the RPE of rats fed different amounts of vitamin A (23, 2.3, .23, .058 and 0.0 mg retinol/kg diet) showed a dose-related response with respect to the amount of lipofuscin accumulated. Thus, for the first time, it appears that vitamin A has a direct involvement in lipofuscin formation, and even may become included in lipofuscin granules as one of the oxidized byproducts of cell metabolism.

Project Description:

Objectives: To study the role of dietary vitamin A in ocular aging as well as in visual function and the maintenance of the retina. This is part of our continuing effort to examine what specific functions of the pigment epithelial cells are altered or lacking under various experimental and pathological conditions which might influence their ability to provide proper maintenance of the visual apparatus.

Methods Employed: We designed an experiment to determine the possible involvement of vitamin A as well as retinal lipids containing many polyunsaturated fatty acids in the formation of lipofuscin in the retinal pigment epithelium. Weanling albino rats, females of the Sprague-Dawley strain, were divided into 5 groups and fed purified diets free of vitamin A or containing .058, .23, 2.3, or 23 mg retinol/kg diet, respectively. After 18 and 30 weeks on the diets, the pigment epithelial cells were examined in frozen sections by fluorescence microscopy and in embedded sections by light and electron microscopy. The intensity of lipofuscin-specific autofluorescence was recorded photographically, and the number of PAS-positive granules were counted in photomicrographs.

Major Findings: A close relationship between vitamin A and lipofuscin formation was demonstrated by a dose-related response to dietary vitamin A. Rats receiving increasing amounts of dietary vitamin A had increasingly more lipofuscin in their retinal pigment epithelium, as demonstrated both by autofluorescence and by granule counts.

Significance to Biomedical Research and the Program of the Institute: Vitamin A (retinol) has a central role in the visual process and undergoes dynamic exchange between the photoreceptor cells and the retinal pigment epithelium upon light adaptation and during the daily cycle. The pigment epithelium contains more than 90% of the vitamin A stores of the retina and these occur in the form of lipid droplets, as we showed previously.

The present results support the notion that the pigment epithelium normally processes many breakdown products of retinol as well as the lipids of disk membranes, and that greater availability of retinol results in more of its breakdown products being incorporated into lipofuscin. This could explain why rats which lack rod outer segments still accumulate much lipofuscin when retinol is present. Retinol, like the fatty acids of outer segments, contains many double bonds. Besides, its double bonds are conjugated, very excitable by light, and quite unstable unless protected from oxygen. Therefore, the additional lipofuscin which accumulates in retinas of rats receiving high levels of dietary vitamin A may contain high amounts of highly oxidized vitamin A products, and the lipofuscin in normal rat and human retinas may contain substantial amounts.

Proposed Course: We plan to expand our preliminary attempts to reverse lipofuscin (aging pigment) formation in the retinal pigment epithelium by using the drug centrophenoxyne which has been shown to decrease the amount of lipofuscin accumulated in the central nervous system and to restore functions lost through such accumulation.

NEI Research Program: Retinal and Choroidal Diseases--Retinal Pigment Epithelium

Publications:

Datiles M, Hu T-S, Kador P, Robison WG Jr, Kinoshita J: Glucose levels related to recovery from scraping. Invest Ophthalmol Vis Sci 22(suppl): 25, 1982.

Robison WG Jr, Katz ML, Bieri JG: Eye lipofuscin response to the "aging-reversal" drug centrophenoxine. Invest Ophthalmol Vis Sci 22 (suppl):63, 1982.

Gery I, Shichi W, Robison WG Jr, El-Saied M, Nussenblatt RB: Transfer of experimental autoimmune uveitis (EAU) by cultured lymphocytes. Invest Ophthalmol Vis Sci 22(suppl):213, 1982.

Carter-Dawson L, Wiggert B, Robison WG Jr: Potential role of the 7S retinol-binding protein in vision. Invest Ophthalmol Vis Sci 22(suppl):249, 1982.

McKenna MC, Robison WG Jr, Bieri JG: Cellular localization of liver vitamin A in rats given total parenteral nutrition (TPN) solutions intravenously or orally. Fed Proc 41:387, 1982.

Robison WG Jr, Kuwabara T, Zwaan J: Eye research, Chapter 5, in Foster HL, Small JP, Fox J (eds): The mouse in Biomedical Research Vol 4. New York, Academic Press, 1982.

Robison WG Jr, Kuwabara T, Bieri JG: The roles of vitamin E and unsaturated fatty acids in the visual process, in Dowling JE (ed): Symposium on Nutrition, Pharmacology and Vision, The Retina, 1982.

Robison WG Jr, Katz ML, Bieri JG: Lipofuscin response to the "aging-reversal" drug centrophenoxine. I. Vitamin E-related lipofuscin in rat eye, uterus, and brain. Invest Ophthalmol Vis Sci (in press).

Katz ML, Robison WG Jr: Lipofuscin response to the "aging-reversal" drug centrophenoxine. II. Age-related lipofuscin in rat eye, uterus, and brain. Invest Ophthalmol Vis Sci (in press).

PERIOD COVERED

October 1, 1981, to May 12, 1982

TITLE OF PROJECT (80 characters or less)

Role of Vitamin A in Maintenance and Development of Ocular Tissues

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Louvenia Carter-Dawson	Ph.D.	Staff Fellow	LVR	NEI
Other:	W. Gerald Robison, Jr.	Ph.D.	Chief, Section on Experimental Anatomy	LVR	NEI
	Toichiro Kuwabara	M.D.	Chief, Section on Experimental Pathology	LVR	NEI
	Barbara Wiggert	Ph.D.	Research Chemist	LVR	NEI
	John G. Bieri	Ph.D.	Chief, Section on Nutritional Biochemistry	LNE	NIADDK

COOPERATING UNITS (if any)

Laboratory of Nutrition and Endocrinology, NIADDK

LAB/BRANCH

Laboratory of Vision Research

SECTION

Section on Experimental Anatomy

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

1.3

PROFESSIONAL:

1.2

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

 (a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER (a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The neural retina has a soluble 7S retinol-binding protein which was described previously (Wiggert et al., 1976). To elucidate more precisely the role of this protein in vision we examined developmental changes in the amounts of tritiated retinol bound to the 7S protein and compared these to rhodopsin levels and retinal structure in the C57BL/6 mouse. Binding of tritiated retinol by the 7S protein is not detected in retinal cytosol until the 13th postnatal day (P13). Approximately 0.4 pmoles of retinol/mg cytosol protein is bound at P13. A 6-10 fold increase in amount of retinol bound is seen over the next seven days reaching adult levels of 9-12 pmoles of retinol bound/mg cytosol protein around P40. The initial detection of tritiated retinol binding to the 7S protein does not coincide with the appearance of outer segments or rhodopsin which are detectable at P5 and P7 respectively. However, detection of tritiated retinol binding to this protein does coincide with eye opening at which time bleaching of rhodopsin occurs. The detection of the 7S retinol-binding protein at this stage of visual maturation suggests it may play a role in the cycle of rhodopsin bleaching and regeneration.

Project Description:

Objectives: It is clear that vitamin A (retinol) is involved in the visual cycle, but just how it is transported between the neural retina and retinal pigment epithelium and how its concentration is maintained between bleaching and regeneration of rhodopsin is not fully determined. This project was designed to investigate the possible role of a retinol-binding transport protein in the visual process.

Methods Employed: Cytosol preparations were made by homogenizing neural retinas of male C57BL/6 mice at various postnatal ages (8-300 days). Following incubation with tritiated retinol the binding proteins were separated by sucrose density gradient centrifugation. Rhodopsin levels were determined by absorption spectra before and after light exposure. Protein analyses were done using the Lowry method. Light and electron microscopy were utilized to document changes in rod outer segment structure and to look for correlations with the biochemical changes.

Major Findings: The developmental appearance of the 7S retinol-binding protein correlated with eye opening. It did not correlate with the early postnatal development of rod outer segments or with the appearance of rhodopsin.

Significance to Biomedical Research and the Program of the Institute: Since the 7S retinol-binding protein first appears at the time of eye opening, it may be involved in changes which occur between dark- and light-adaptation. It may play a role in the cycle of bleaching and regeneration of rhodopsin. Such studies on the basic mechanisms of the visual process can provide information that may be useful in identifying human disorders which may involve defects associated with storage, utilization, or uptake of this vitamin during the development of ocular tissues.

Proposed Course: Attempts will be made to determine if the 7S retinol-binding protein binds different amounts of retinol in the dark than in the light, and experiments will be designed to find out whether this protein is intracellular or extracellular. This will be attempted as a collaborative project between Dr. Wiggert at the NEI and Dr. Carter-Dawson who now is at the University of Texas Health Science Center in Houston working with Dr. Harry G. Sperling.

NEI Research Program: Retinal and Choroidal Diseases--Toxic, Nutritional, and Environmental Disorders

Publications:

Carter-Dawson L, Kuwabara T, Bieri JG: Intrinsic, light-independent, regional differences in photoreceptor-cell degeneration in vitamin A-deficient rat retinas. Invest Ophthalmol Vis Sci 22:249-252, 1982.

Carter-Dawson L, Wiggert B, Robison WG Jr: Potential role of the 7S retinol-binding protein in vision. Invest Ophthalmol Vis Sci 22(suppl): 249, 1982.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 EY 00145-01 LVR

PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Effects of Aging and Nutrition on the Retina and Retinal Pigment Epithelium

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Martin L. Katz	Ph.D.	Staff Fellow	LVR NEI
Other:	W. Gerald Robison, Jr.	Ph.D.	Chief, Section on Experimental Anatomy	LVR NEI

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Vision Research

SECTION

Section on Experimental Anatomy

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.4	.2

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Investigations were carried out to characterize senescent changes in the pigmented rat retina and retinal pigment epithelium (RPE) using morphological and biochemical techniques. Age related losses of cells from the photoreceptor, inner nuclear, and ganglion cell layers were found. Rhodopsin levels were found to change very little during aging. Senescent changes in the RPE included: (1) a progressive accumulation of lipofuscin; (2) a shortening and thickening of the apical microvilli; (3) modification and enlargement of the basal infoldings; and (4) accumulation of electron-dense material between the basal infoldings. We have found that the drug centrophenoxine does not reverse age-related lipofuscin accumulation in the RPE. Acid phosphatase levels in the RPE did not change during senescence. We are currently performing measurements to determine whether aging has an affect on photoreceptor disc shedding and phagocytosis by the RPE. We will also determine whether vitamin A uptake and esterification by the RPE during light adaptation are altered during senescence. Finally, we are characterizing the apical membrane proteins of the RPE, and will determine whether these proteins are altered with age.

Project Description:

Objectives: To study age related changes in the retina and retinal pigment epithelium (RPE) and determine the mechanisms underlying these changes. In addition, we are evaluating various potential means for halting or reversing senescent changes in these tissues.

Methods Employed: We are examining various structural and functional parameters in the eyes of pigmented rats of various ages. Age related morphological changes are being characterized using fluorescence and bright-field light microscopy, as well as electron microscopy. The effects of senescence on rhodopsin and certain enzyme levels are being measured by spectrophotometric techniques. Vitamin A exchange between the retina and RPE during light adaptation is being measured using high pressure liquid chromatography. The potential effect of the drug centrophenoxine in reversing age changes in some of these parameters is being examined.

Major Findings: There is a progressive loss of photoreceptor cells from the retina during senescence, the rate of cell loss being almost constant in rats between 4 and 32 months of age. Despite an almost 30% loss of photoreceptors over this age span, the amount of rhodopsin per eye remains almost constant, indicating that the amount of visual pigment per cell must rise during aging. Very little age related cell loss occurs from the inner nuclear layer (INL) between 4 and 26 months of age. However, between 26 and 32 months of age, there is a 25% loss of INL cells. Significant loss of cells from the ganglion cell layer (GCL) of the retina occurs only between the ages of 4 and 11 months, during which 30% of the cells disappear from this layer. No further loss of cells from the GCL occurs up to 32 months of age.

In order to determine whether primary changes in the RPE might be responsible for the observed loss of photoreceptors with age, ultrastructural analysis was carried out on the RPE of rats of various ages. Senescent changes in the RPE included: (1) a progressive accumulation of lipofuscin; (2) a shortening and thickening of the apical microvilli; (3) modification and enlargement of the basal infoldings; and (4) accumulation of electron-dense material between the basal infoldings.

In order to determine whether the ability of the RPE to degrade phagocytized photoreceptor outer segments is impaired during senescence, the activity of a lysosomal marker enzyme, acid phosphatase, was measured in these cells in rats of various ages. No age related changes in acid phosphatase levels were seen between 4 and 32 months of age.

It is possible that the age related accumulation of lipofuscin in the RPE leads to an impairment of cell function. The drug centrophenoxine has been reported to reverse the accumulation of age related lipofuscin in the central nervous system of a variety of species. We treated two year old Fisher rats with centrophenoxine for 11 weeks and found that it had no effect on RPE lipofuscin content. Contrary to previous reports, we found that centrophenoxine also failed to reverse age related lipofuscin accumulation in the brain.

Significance to Biomedical Research and the Program of the Institute: In the U.S. today, senile changes of the retina are among the most prevalent causes of serious visual impairment. Several reports indicate that the incidence of senile macular degeneration (SMD) reaches about 30% in the population between 75 and 85 years of age. This problem is likely to increase dramatically in the current decade, as more people survive in older age. In many cases of SMD, the visual disturbances require complete and perpetual institutional care of the patient, compromising the quality of life of the patient and placing emotional and financial burdens on families and society. Through our studies, we hope to gain an understanding of the fundamental processes underlying age changes in the retina. Ultimately, we hope to develop a rational approach for preventing the development of degenerative retinal changes in the elderly.

Proposed Course: We plan to continue our studies to define precisely age related changes in the structure and function of the retina and RPE. Particular attention will be focused on characterizing senescent changes in the interactions between the retina and RPE. We will determine whether aging affects the ability of the RPE to assimilate and esterify retinol released from the rod outer segments during light adaptation. In addition, we will determine whether senescence has an affect on rod outer segment disc shedding and phagocytosis by the RPE. We are also currently attempting to characterize the apical membrane proteins of the RPE which may mediate RPE-retina interactions. Finally, we will conduct further experiments to evaluate the potential role of RPE lipofuscin accumulation in the development of senile changes in the retina.

NEI Research Program: Retinal and Choroidal Diseases--Retinal Pigment Epithelium

Publications:

Katz ML, Parker KR, Handelman GJ, Bramel TL, Dratz EA: Effects of anti-oxidant nutrient deficiency on the retina and retinal pigment epithelium of albino rats: a light and electron microscopic study. Exp Eye Res 34: 339-369, 1982.

Katz ML, Handelman GJ, Parker KR, Dratz EA: Structural and biochemical effects of antioxidant nutrient deficiency on the rat retina and retinal pigment epithelium. Ann NY Acad Sci (in press).

Robison WG Jr, Katz ML, Bieri JG: Eye lipofuscin response to the "aging reversal" drug centrophenoxine. Invest Ophthalmol Vis Sci 22 (ARVO suppl):63, 1982.

Robison WG Jr, Katz ML, Bieri JG: Lipofuscin response to the "aging-reversal" drug centrophenoxine. I. Vitamin E-related lipofuscin in rat eye, uterus, and brain. Invest Ophthalmol Vis Sci (in press).

Katz ML, Robison WG Jr: Lipofuscin response to the "aging-reversal" drug centrophenoxine. II. Age-related lipofuscin in rat eye, uterus, and brain. Invest Ophthalmol Vis Sci (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00005-10 LVR

PERIOD COVERED October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Electrophysiology and Morphology of Mammalian and Avian Retinas

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Ralph Nelson	Ph.D.	Physiologist	LVR	NEI
	Avery Nelson	Ph.D.	Senior Staff Fellow	LVR	NEI
	Andrew Mariani	Ph.D.	Staff Fellow	LVR	NEI

COOPERATING UNITS (if any)

Department of Physiology, University of Utah, Salt Lake City
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LAB/BRANCH
Laboratory of Vision Research

SECTION
Section on Experimental Biology

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	2.5	PROFESSIONAL:	2.5	OTHER:	0.0
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CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We study the interactions among and interconnections between retinal neurons using electrophysiological and anatomical techniques. Neurons in cat, monkey, or pigeon retina are stained by the Golgi technique or by direct, intracellular injection of horseradish peroxidase (HRP) and subsequent histochemical reactions. The extents of dendritic and axonal arborizations of morphologically identified cells are illustrated in camera lucida drawings. Electrophysiological response properties are determined by intracellular recording. Electron microscopy reveals the synaptic relationships between photoreceptor, horizontal, bipolar, amacrine, and ganglion cell types. These data allow the inference of neural circuits and functional units within the retina and demonstrate the correlations between neural structure and function.

Project Description:

Objectives: To understand the functional, structural, and ultrastructural organization of mammalian and avian retinas; to discover the synaptic interconnections among neurons and the functional pathways between them; and to elucidate retinal function in normal and diseased states.

Methods Employed: Golgi impregnation which stains widely-separated individual cells in their entirety is used to study the form, shape, size, and spatial relationships of retinal neurons. Their synaptic connections with other neurons are then examined by Golgi-EM, thus providing insight into their function.

We characterize the response properties of neurons in retinas by intracellular recording of their transmembrane potentials and extracellular electroretinographic (ERG) recording during photic stimulation. Viable retina-eyecup preparations are maintained *in vitro* by perfusion of the ophthalmic arteries and retinal surface with synthetic media. HRP, injected into neurons through intracellular electrodes, fills their axons and dendrites, and the morphology of individual, physiologically studied neurons is revealed in the light and electron microscope. In the light microscope, cells are drawn and classified according to analogy with their Golgi counterparts; in the electron microscope, the synapses forming neural input and output can be identified. Receptive field properties of neurons and the extent to which they are physiologically activated by rod or different cone mechanisms can be determined from analysis of the electrical responses. Thus, the synaptic relationships of physiologically studied cells with other retinal neurons can be known and the retinal pathways along which visual information travels can be revealed.

Major findings: I. Electron microscopy and electrophysiology of bipolar cells in the cat retina. The division of the cat inner plexiform layer (IPL) into separate layers for on- and off-center ganglion cells is one of its most profound aspects. It is thus pertinent to inquire about bipolar input to these separate layers and the nature of the contacts these bipolar cells make with photoreceptors. We have been able to measure the response properties of several different bipolar cell types in the cat retina and to examine their contacts with photoreceptors and ganglion cells. Two types of invaginating bipolars (the rod bipolar and the cone bipolar cb6) have axons arborizing in layer b of the IPL, among the dendrites of on-center ganglion cells. Both of these were center hyperpolarizing. Cb6 contacts on-center ganglion cells directly. One flat cone bipolar (cb2) whose axon arborizes in layer a among the dendrites of off-center ganglion cells with which it makes direct contact, proved also to be center hyperpolarizing. Some bipolar cells proved to make yet another sort of receptor contact. This has been termed "the semi-invaginating basal junction". Such contacts occur in association with the ribbon synaptic complex of cones. A bipolar dendrite invaginates the base of a cone pedicle, makes a basal junction, but does not closely approach the arciform density of the ribbon. Usually a dendrite from another cone bipolar occupies the central position of the triad. One bipolar cell that contacts cones with the semi-invaginating basal junction is cb5, whose axon terminal branches in layer b among the dendrites of on-center ganglion cells with which it makes frequent synaptic contact. Cb5 is a depolarizing bipolar. Cb1, whose

axon terminal contacts the dendrites of off-center ganglion cells (in layer a of the IPL) makes a morphologically similar receptor contact, but its response properties are not known. Clearly, our understanding of cat bipolar cells is still incomplete, but these findings suggest that these cells may be much more elaborate in their receptor contacts and in their connectivity with amacrine and ganglion cells than previously suspected. In particular, the center sign of ganglion cell responses cannot simply be related to the flat and invaginating distinction in photoreceptor-bipolar contacts or to the center sign of bipolar cells branching in layers a and b of the IPL.

II. A bipolar cell type in rhesus monkey retina selective for short-wavelength sensitive cones. In Golgi preparations of the rhesus monkey (Macaca mulatta) retina, a new bipolar cell type which selectively contacts a small population of cones has been identified. These bipolar cells have somata in the middle to outer one-half of the inner nuclear layer and several primary dendrites, some of which converge at a distance from the axis of the cell body to form a cluster of large terminals at the level of the cone pedicles, while the other dendrites end wholly within the inner part of the inner stratum of the outer plexiform layer and do not contact any photoreceptor terminal. Their axons end as large, narrowly stratified terminals in the innermost stratum of the inner plexiform layer. Since the dendritic and axon terminal spans of this cell type are much larger than midget bipolar cells, but like midgets usually contact only a single cone, it is termed a "single cone contacting, non-midget" bipolar cell. Golgi-E.M. shows that these cells form central elements of triads at the ribbon synaptic complex of cone pedicles and, distal to the ribbons, form narrow-cleft junctions with portions of the cone pedicles. Like midget bipolar cells, the "single cone contacting, non-midgets" occasionally contact two cones, but the two cones are widely separated, not adjacent as in the case of double-midgets. By relating the intercone distances of the two cones contacted by these newly identified bipolar cells to a percentage of the total cone population, their connection with a specific spectral class of cone can be surmised. For example, at 3 mm eccentricity where there are 6400 cones/mm sq., the intercone distance of the contacts of this selective bipolar cell is 32 μ m which is 14% of the cones. This figure and those at all retinal locations measured are in good agreement with histochemical staining methods for blue cones in monkeys. Thus, it appears that the "single cone contacting, non-midget" bipolar cells of rhesus monkey retina are selective for blue cones.

III. Association amacrine cells could mediate directional selectivity in pigeon retina. Ganglion cell receptive fields in the vertebrate retina are classified as "simple" if they display mutually antagonistic, concentric on and off responses to stimulation by light, or as "complex" if they respond maximally to a stimulus which moves in a certain direction or has a particular orientation. One "complex" unit frequently reported is directionally selective. It is excited by stimuli moving through its receptive field in only one direction, the preferred direction. Motion in the reverse or null direction inhibits these units. In species such as cats and monkeys which have predominantly "simple" center-surround organization of ganglion cell receptive fields, there is a low ratio of conventional (amacrine cell) to ribbon (bipolar cell) synapses in the inner plexiform layer, while in species such as pigeons and frogs which have a large percentage of "complex" ganglion cell receptive fields, there is a high ratio of conventional to ribbon (amacrine cell to

bipolar cell) synapses. Therefore, it is thought that amacrine cells, which are laterally interconnecting neurons in the inner plexiform layer, form the "complex" ganglion cell receptive fields.

Although quantitative electron microscopic studies of the inner plexiform layer indicate that amacrine cells may form the complex receptive field properties of ganglion cells, they offer no clues as to the particular morphology of amacrine cells involved. In pigeon retina, the amacrine cells were studied using Golgi impregnation in both whole-mounted and radially sectioned material to see if there may be a correlation between the morphology of amacrine cells and the receptive fields of ganglion cells.

Amacrine cells of the pigeon retina can be divided into at least two classes based on their morphology as observed in whole-mounted Golgi preparations. Class I amacrine cells are typical of vertebrate amacrines in that they have dendrites which display a radial symmetry and, as their name implies, lack axons. Class II amacrine cells, however, clearly have an intraretinal axon and terminal arborization in addition to the dendrites radiating from their cell bodies. These "association" amacrine cells are thus morphologically polarized, and this polarized organization is consistent with the physiological features of the directionally selective receptive fields of ganglion cells recorded in pigeon retina.

IV. Intracellular recordings from a bplexiform cell in macaque retina. A bplexiform cell has been penetrated with an HRP-filled microelectrode in the retina of Macaca fascicularis. With background and stimulus conditions favoring rods, which such cells are known to contact directly, on-off, depolarizing, L-type waveforms were evoked which had a rapid onset and slow decay. With rod suppressing blue backgrounds (631,000 td), rectangular, L-type depolarizations remained, driven at least by one longwavelength cone type. The cell body, located in the GCL 2 mm from the fovea, was 9 μm in diameter and surrounded by a few loose wavy dendrites spanning 70 to 100 μm in the innermost IPL. One of several processes ascending through the IPL and INL, could be traced to the layer of rod spherules. A fine, curvy axon with loops and a single spine coursed 0.3 mm through the mid-IPL before descending abruptly to the optic nerve fiber layer, turning and traveling 2 mm directly to the optic disk. It was less than 1 μm in diameter except for regularly spaced varicosities. On-off cells of the retina are thought to receive inputs from both on- and off- bipolar pathways. The mammalian rod system, with only one type of bipolar cell, presents a unique problem for the formation of an on-off receptive field. The bplexiform cell may solve this problem by obtaining one of its bipolar inputs from the rod bipolars, while tapping the rods directly for the other. In the presence of neutral backgrounds common to extracellular recording, it seems likely however that this cell would be classified as a broad-field, sustained, L-type unit.

Significance to Biomedical Research and the Program of the Institute: In diagnosing and treating the diseases of the eye it should prove useful to understand retinal function at the cellular level and the pathways through which visual signals travel and are processed. In this regard it is interesting that our repertoire of intracellularly studied and stained neurons now includes several from cats afflicted with central retinal degeneration. These are not in sufficient quantities as yet for us to draw conclusions concerning modifications of retinal pathways. All amacrine cells of cat retina mediate the ability to perceive small dim objects quickly. It would be

interesting if deficits of this kind were observed clinically. Our recent discovery in the cat retina of the extreme sensitivity of the ERG b-wave to the spatial pattern of the stimulus now has been demonstrated in humans. Our spatial stimulus protocol appears uniquely suited to assessing image quality in peripheral retina and thus may have clinical relevance. In the inner plexiform layer of macaques, the blue system has been localized to a narrow stratum close to ganglion cell bodies. This location may make it particularly susceptible to specific sorts of injuries. A knowledge of what neurons the retina contains, their morphological, physiological, and chemical properties and their roles in vision provides a necessary substrate for interpreting, testing, and treating retinal dysfunction.

Proposed Course: This project will be continued along the lines indicated in the project description. Emphasis will be given to studying the interconnections and interactions among neurons participating in the inner and outer plexiform layers of primates and cats using electrophysiological, anatomical, and biochemical techniques.

NEI Research Program: Retinal and Choroidal Diseases--Retinal Organization, Neurotransmission, and Adaptation.

Publications:

Nelson R: All amacrine cells quicken the time course of rod signals in the cat retina. J Neurophysiol 47:928-947, 1982.

Nelson R, Kolb H, Robinson M, Mariani A: Neural circuitry of the cat retina: Cone pathways to ganglion cells. Vision Res 21:1527-1536, 1981.

Dickinson-Nelson A, Reese TS: Structural changes during transmitter release at synapses in the frog sympathetic ganglion. J Neurosci (in press)

Kolb H, Nelson R: Rod pathways in the retina of the cat. Vision Res (in press).

Nelson R, Kolb H: Synaptic patterns and response properties of bipolar and ganglion cells in the cat retina. Vision Res (in press).

Mariani AP: Biplexiform cells: Ganglion cells of the primate retina that contact photoreceptors. Science 216:1134-1136, 1982.

Nelson R, Kolb, H: Synaptic patterns and response properties of bipolar and ganglion cells in the cat retina. Invest Ophthalmol Vis Sci 22 (suppl):175, 1982.

Zrenner E, Nelson R, Mariani AP: Intracellular recordings from a biplexiform cell in macaque retina. Invest Ophthalmol Vis Sci 22(suppl): 279, 1982.

Project No. Z01 EY 00005-10 LVR

Mariani AP: Newly identified bipolar cells in monkey retina. Invest Ophthalmol Vis Sci 22(suppl):247, 1982.

Mariani AP: Association amacrine cells could mediate directional selectivity in pigeon retina. Nature 298:654-655, 1982.

Kolb A, Nelson R: Amacrine cells of the cat retina. Vision Res 21:1625-1633, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00125-02 LVR

PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Neuropharmacology of the Retina

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Donald G. Puro	M.D., Ph.D.	Medical Officer Ophthalmologist	LVR NEI
Other:	Hermes H. Yeh Barbara-Anne Battelle	Ph.D. Ph.D.	Staff Fellow Senior Staff Fellow	CB NEI LVR NEI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Vision ResearchSECTION
Section on Experimental BiologyINSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, MD 20205

TOTAL MANYEARS: 2.0 PROFESSIONAL: 2.0 OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

 (a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER (a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Knowledge basic to the development of a pharmacological approach to the prevention and treatment of retinal disorders is being acquired. A combination of technological advances in neuropharmacology, intracellular electrophysiology and cell biology, including cell culture, is used to explore the actions and interactions of neurotransmitters, neuromodulators, hormones and selected drugs on specific types of retinal neurons.

Project Description:

Objectives: The long range goal of this project is to find pharmacological tools that can be used to prevent or treat retinal disorders. The strategy for achieving this goal is to identify and investigate molecules that influence the growth and function of retinal neurons. Knowledge of various regulatory molecules should lead to the discovery of chemotherapeutic agents which could do such things as make injured retinal circuits work better or rescue neurons of the retina before they become irreversibly damaged.

Specific, short term objectives are to apply technical advances in electrophysiology, neuropharmacology and cell biology, including cell culture, in order to: (1) explore the effects of neurotransmitters, neuromodulators, hormones, and selected drugs on the function of specific types of retinal neurons, (2) discover molecules which regulate the functional maturation of neurons of the retina; and (3) establish parameters for assaying the effects of therapeutic and toxic agents on developing retinal neurons.

Methods Employed: Using techniques of intracellular electrophysiology and cell biology, we have developed a cell culture system to study acetylcholine-synthesizing neurons of the rat and chick retina. The synaptic release of neurotransmitter from a single, visually identified, cholinergic neuron can be monitored continuously using this system. Putative neurotransmitters, neuromodulators, and drugs are applied near a cholinergic neuron by microiontophoresis or pressure ejection from micropipets.

Major Findings: (1) Hormones may play a vital role in the functional maturation of retinal neurons. Glucocorticoid steroid hormones were found to regulate the developmental step in which a retinal neuron becomes capable of information transfer. This hormonal effect occurs at physiological concentrations, is mediated by glucocorticoid receptors, acts at the transcriptional level and affects the depolarization-coupled release of neurotransmitter. Findings using our cell culture system led to in vivo studies. We discovered that injecting pregnant rats with dexamethasone, a synthetic glucocorticoid, accelerates the functional maturation of retinal neurons of the fetus. Of further interest, maternal stress, which elevates glucocorticoid levels, also causes this precocious functional development. These studies raise the possibility that an alteration of synaptic maturation secondary to an elevation of maternal glucocorticoid levels due to hormone administration, stress, or disease is an etiology of certain developmental disorders of the retina.

(2) Dopamine, a putative retinal neurotransmitter, can regulate how a cholinergic neuron responds to excitatory stimuli. Using our cell culture system, we found that a brief exposure to dopamine has a long-term influence on the ability of a cholinergic neuron to transmit excitatory information to a postsynaptic target. Using techniques that we have recently developed, the effects of dopamine on the release of neurotransmitter from a single, visually identified cholinergic retinal neuron can be continuously monitored. This provides a unique system to analyze further the effects of dopamine on neurons of the mammalian retina. Identification and investigation of a neuro-

transmitter that may "tune" the responsiveness of retinal neurons is important since manipulation of such a neurotransmitter system could have potential therapeutic uses in the modification of injured retinal circuits.

(3) It was discovered that cyclic AMP may regulate the function of retinal neurons. Analogs of cyclic AMP enhanced the ability of embryonic cholinergic of the rat retina to release acetylcholine at synapses in response to excitatory stimulation. This effect was mimicked by a phosphodiesterase inhibitor. We are exploring the concept that cyclic AMP is a vital link between metabolic events at the neuronal surface and intracellular processes which are important in growth and function. This strategic position of cyclic AMP may make pharmacological manipulation of its intracellular levels a potent clinical tool in the future.

(4) Two potentially vulnerable periods in the maturation of cholinergic neurons of the rat retina were identified in an electrophysiological and biochemical analysis (see also Z01 EY 00066-03 LVR). One period is early in retinal morphogenesis before synaptic specializations appear. Even at this early stage, many differentiated properties, including the ability to release acetylcholine onto postsynaptic cells, can be expressed by cholinergic neurons. A second period occurs later, concomitant with morphological signs of synaptogenesis. The functions quantitatively described in this study serve as parameters for the detection of effects of drugs on developing retinal neurons. For example, we found that cholinergic neurons are vulnerable to exposure to a synthetic hormone, dexamethasone, during the early developmental period.

Significance to Biomedical Research and the Program of the Institute:
Retinal disorders are a major cause of irreversible visual loss. To devise ways to prevent or treat retinal problems, a greater understanding of pathophysiology and pharmacology at the cellular level is required. The combination of technological advances on electrophysiology, neuropharmacology, and cell biology has led us to the development of methods for exploring the function of a single retinal neuron. We have discovered actions of hormones, neurotransmitters, and other molecules that ultimately may prove useful in the development of pharmacological tools to combat retinal disorders.

Proposed Course: Emphasis will be placed on the study of interactions of neurotransmitters and neuromodulators at the neuronal level. Our recent finding that insulin may have a role in the function of embryonic retinal neurons will be vigorously pursued. The multidisciplinary nature of the project will be extended with collaborative work using techniques in biochemistry, electron microscopy, histofluorescence, and autoradiography. Recent advances in biophysics which allow the study of single ion-channels will be implemented.

NEI Research Program: Retinal and Choroidal Diseases--Retinal Organization, Neurotransmission, and Adaptation

Publications:

Puro DG, Battelle B-A, Hansmann KE: Development of cholinergic neurons of the rat retina. Develop Biol 91:138-148, 1982.

Battelle B-A, Puro DG, Hansmann KE: Early development of cholinergic function in rat retina. Soc Neurosci 7:399, 1981.

Puro DG: Classes of cholinergic retinal neurons in cell culture. Soc Neurosci 7:277, 1981.

Puro DG: Glucocorticoids alter the functional maturation of cholinergic retinal neurons. Invest Ophthalmol Vis Sci 22(suppl):81, 1981.

Yeh HH, Puro DG: Cyclic AMP regulates the development of neurotransmission in cultured retinal neurons. Invest Ophthalmol Vis Sci 22(suppl):278, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00012-03 LVR
PERIOD COVERED October 1, 1981 to September 30, 1982			
TITLE OF PROJECT (80 characters or less) Growth of the Retinal Pigment Eptiehlium			
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT			
PI: Alfred J. Coulombre Ph.D.		Chief, Section on Experimental Embryology	LVR NEI
Other: None			
COOPERATING UNITS (if any)			
LAB/BRANCH Laboratory of Vision Research			
SECTION Section on Experimental Embryology			
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205			
TOTAL MANYEARS:		PROFESSIONAL:	OTHER:
1.0		1.0	0.0
CHECK APPROPRIATE BOX(ES)			
<input type="checkbox"/> (a) HUMAN SUBJECTS		<input type="checkbox"/> (b) HUMAN TISSUES	
<input checked="" type="checkbox"/> (c) NEITHER			
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS			
SUMMARY OF WORK (200 words or less - underline keywords)			
<p>The formation of <u>neural retina</u> (NR) from <u>retinal pigmented epithelium</u> (RPE) of chick embryos in culture was investigated. In cultures of explants of RPE, depigmented, <u>preretinal foci</u>, consisting of 50 to 100 cells appeared in the pigmented central portion of the explant within three days. Then these <u>depigmented cells</u> increased rapidly in number and by about day 14 they formed characteristic spherical bodies, which were identified as a neural retinal-like structure (NR structure) by electron microscopic observations. Culture of explants of RPE from embryos of different stages showed that the capacity of embryonic RPE to form an NR structure decreased steadily with embryonic age from stage 24 to 27. At and after stage 27, no foci leading to the <u>neural retinal differentiation</u> were formed in the explants. Medium conditioned by cell cultures of chicken embryonic NR, RPE, or chondrocytes had no effect on the formation of NR structures by explants of RPE.</p>			

Project Description:

Objectives: A switch in the differentiated state of once-specialized eye tissues has been demonstrated in tissue and cell cultures *in vitro*. Cells of the retinal pigmented epithelium of both chick embryos and adult newts differentiate into lens cells in long-term cell cultures *in vitro*. Embryonic chick neural retinal cells (NR cells) form lens and/or pigmented cells *in vitro*. These findings offer a new approach to studies of the stability of the differentiated state as well as the mechanisms of differentiation. In addition, it is well known, particularly from *in vivo* studies on regeneration of ocular tissues in both chick and amphibians, that RPE has the capacity to form neural retina (NR) in developing eyes.

Transdifferentiation of NR from RPE, however, has not yet been demonstrated in culture although there is a preliminary report of differentiation of NR from RPE of adult newt in cell culture. In this report, we present evidence that RPE of chick embryos cultured *in vitro* can form a structure that is similar in many respects to the neural retina.

Methods Employed: Staged embryos of white Leghorn chickens, reared at 37.5 degrees in a forced-draft incubator, are used in this investigation. Usually 4 to 6 explants of RPE, squares of about 0.4 to 1.0 cm, were transferred by pipette to each 35-mm Falcon plastic culture dish with 3 ml of Eagle's essential medium (MEM; Nissui, Tokyo, Japan) supplemented with 10% fetal calf serum (Gibco). Explants were attached to the culture substrate of the dish by forcing the edges of the explant into the plastic substrate with forceps to prevent infolding or detachment. Care was taken to place the basal side of the RPE facing the substrate. Explants were maintained at 37.5 degrees, centigrade in a humidified atmosphere of carbon dioxide:air at pH 7.2. The medium was replaced every 3 days. At the end of the culture period, the total area of each explant was measured by planimetry from camera lucida tracings.

Major Findings: In the present culture experiments, the first visible step in transdifferentiation of the NR structure was the appearance of depigmented foci in isolated pieces of the RPE. There is no conclusive evidence to indicate that each focus consists of a clonal population originating from a single founder cell, though this seems likely. Hypothetically, we can assume the presence of two types of cell populations in the tapetum of early embryos, one consisting of unstable cells that can be switched to form NR under culture conditions, the other consisting of stable cells that remain unchanged.

Our results demonstrated that the number of foci per unit area of tissue in culture decreased with development of donor embryos between stage 24 and 27. Therefore, it is possible that loss of the capacity for regeneration is correlated with a decrease in the number of the hypothetical unstable cells in a given cell population. The finding of Zimmerman seems to be relevant to the change in the capacity of RPE to transdifferentiate into NR. He reported that in chick embryos the rate of melanin synthesis is markedly increased with a decrease of radiolabeled-thymidine uptake and with a striking increase in the generation time in the tapetum between day 4 and 5 of incubation. The stages when cellular changes occur correlate well with the critical stages of decrease in the potentiality of transdifferentiation of these cells detected in the present study.

In the present experiments, lens differentiation also occurred from isolated pieces of RPE. It was recognized, however, only in the peripheral zone of outgrowths of the explants where cells had lost their pigment granules. Only in a few cases, did lenses differentiate from non-pigmented foci formed in the original explant still consisting mostly of pigmented cells. Therefore, these non-pigmented foci may be predominantly competent to form NR, but not competent to form both lens and NR. In some preliminary experiments on cell cultures, however, we observed the formation of lentoid bodies, which were initiated with singly dissociated RPE cells taken from embryos at stage 24. It remains to be determined which external or cellular conditions predispose RPE cells of young chick embryos to differentiate in the direction of either lens or of NR.

Significance to Biomedical Research and the Program of the Institute:
A switch in the stage of differentiation of once-specialized eye tissues has been demonstrated under cell culture conditions in previous studies. In these cases, new differentiations occur after very long lag periods (30 to 100 days), during which the cells replicate repeatedly. In contrast, in the present work, transdifferentiation of the NR structure from RPE, occurred within as little as 7 days. It is noteworthy that many previous studies were made with cells from later embryos (after 8 or 9 days' incubation), whereas donor embryos at 4-6 days of development were used in the present study. Very recently, it has been shown that NR cells from donor embryos at 3-3.5 days of development can differentiate into lens or pigmented cells within 14 days in culture (26, 27, 28). Thus, it is possible that cells of the RPE of these younger embryos, though already specialized in their melanin content and expressing their own phenotypes, are very unstable in the sense that they not only switch easily to other directions of differentiation, but also do so without a long lag. Such switches could occur *in vivo*, dictating normal ocular development or contributing to abnormal development or developmental anomalies in lower animals and in humans.

Proposed Course: This project terminated on 20 August 1982 due to retirement of P.I.

NEI Research Program: Retinal and Choroidal Diseases--Retinal Pigment Epithelium

Publications:

Tsunematsu Y, Coulombre AJ: Demonstration of transdifferentiation of neural retina from pigmented retina in culture. Dev Growth and Differ 23:297-311, 1981.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00023-04 LVR

PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Macrophage Interactions with Other Cells and Their Products

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Manabu Mochizuki	M.D.	Visiting Associate	LVR	NEI
	J. Samuel Zigler, Jr.	Ph.D.	Research Biologist	LVR	NEI
	Robert B. Nussenblatt	M.D.	Head, Section on Ophthalmic Immunology	CB	NEI
	John A. Schmidt	M.D.	Research Associate	LI	NIAID
	Julia Derr	B.S.	Biologist	LVR	NEI

COOPERATING UNITS (if any)

Laboratory of Immunology, NIAID

LAB/BRANCH

Laboratory of Vision Research

SECTION

Section on Experimental Immunology

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.6	1.8	0.8

CHECK APPROPRIATE BOX(ES)

 (a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER (a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Various aspects of macrophage interactions with ocular and other cells were examined. (1) "Activated" macrophages, which are a major component of inflammatory reactions, may affect the metabolism of surrounding tissues. This damaging effect was further studied by using lens epithelial cells as the target. Of importance is the finding that prostaglandins regulate this macrophage activity, without acting directly as the damaging mediators. (2) Two macrophage-made mediators which have similar properties are interleukin 1, which activates lymphocytes, and a factor that stimulates fibroblasts. The possibility that these two factors are identical was previously proposed and supported by some preliminary data. This notion has been further supported by a series of experiments showing that the two mediators have multiple identical physical and biochemical properties. (3) Interleukin 1, which mediates certain immune responses, has been used to examine the mode of action of a newly introduced immunosuppressive drug, cyclosporin A. The drug was found to inhibit strongly certain responses mediated by interleukin 1, but to have minimal effect on other responses stimulated by this mediator.

Project Description:

Objectives: Three issues concerning macrophage (Mφ) interactions with other cells have been studied: (1) The cytostatic activity of Mφ. "Activated" Mφ, a major component of chronic inflammatory infiltration, may affect the metabolism of target cells, including lens epithelial cells. This Mφ activity has been hypothetically related to cataract formation in patients with chronic uveitis (see our FY 1981 report). Mφ damage to lens epithelial cells was further analyzed, particularly with regard to the involvement of prostaglandins (PGs). (2) The effect of interleukin 1 (IL 1) on fibroblasts. IL 1, a Mφ product, is a well-defined stimulant for lymphocytes. Our previously reported data (FY 1981) supported the notion that IL 1 may also stimulate fibroblasts and thus may play a role in the wound healing process. This notion was further tested. (3) Effects of cyclosporin A (CsA) on IL 1-mediated responses. CsA, a newly introduced immunosuppressive drug, is currently being tested in various ocular conditions with suspected immune-mediated etiology. Since IL 1 participates in the mediation of many immune responses, we examined the effect of CsA on activation of lymphocyte cultures by IL 1.

Methods Employed: Inflammatory ("activated") Mφ were collected from peritoneal cavities of mice injected with Corynebacterium parvum and their cytostatic effect was measured by the capacity to reduce the DNA synthesis of murine lens epithelial cells (a line kindly provided by Dr. P. Russell, LVR, NEI). Drugs and tested chemicals were added to the cultures to examine their capacity to modulate the cytostasis. Crude preparations of IL 1 were the supernatants from human monocytes cultured with silica particles. The two tested biological activities, the increase in DNA synthesis in cultures of murine thymocytes and of human skin fibroblasts, were measured following treatment of the crude preparations by heat or proteolytic enzymes. In addition, the two activities were measured and compared in fractions obtained from the crude supernatants by six different procedures: chromatography by Sephadex G-200, phenyl Sepharose, SP-Sephadex and DEAE anion exchange, as well as HPLC size exclusion and iso-electrofocusing. CsA effects on IL 1 activities were tested by adding different doses of the drug to lymphocyte cultures stimulated with IL 1 alone (=direct IL 1 mitogenesis), or in combination with antigens or lectins such as phytohemagglutinin or concanavalin A (=potentiating activity).

Major Findings: The study concerning the damaging effect of activated Mφ on lens epithelial cells has produced the following findings. The Mφ effect may be counteracted in part by cyclooxygenase inhibitors (e.g., aspirin and indomethacin). However, the products of this enzyme, PGs, had no direct inhibitory effect on the target lens epithelial cells. Yet, exogenously added PGs completely abolished the counteracting effect of the cyclooxygenase inhibitors while having no clear effect on the activity of other drugs such as glucocorticoids. These findings indicate that PGs act as regulators of the cytotoxic mechanism, rather than as direct mediators of the damaging effect.

The presumed identity between the lymphocyte activating capacity of IL 1 and the Mφ-made fibroblast stimulatory activity was further verified; a complete correlation was found between the two activities following treatment of the Mφ-made preparations by heat or enzymes, and identical localization of

these activities was found in fractions obtained by the different procedures (see Methods Employed for detail).

Examination of the capacity of CsA to inhibit IL 1-mediated lymphocyte responses revealed a dissociation between the two main effects of IL 1: CsA strongly inhibited reactions in which IL 1 potentiates the response of lymphocytes to antigens or lectins. On the other hand, CsA had minimal effect on the direct mitogenic activity of IL 1 on thymocytes.

Significance to Biomedical Research and the Program of the Institute:
The data concerning the cytostatic effect of Mφ shed new light on the pathogenic effect of these inflammatory cells, particularly with regard to the regulatory role of PGs. In addition, these findings may help to understand better the mode of action of different anti-inflammatory drugs in suppressing the damaging effects of the Mφ. Information of this kind should be particularly important to studies aimed at preventing the irreversible damage inflicted on ocular tissues by inflammatory processes.

The results indicating the identity between IL 1 and the fibroblast stimulating activity add a new dimension to the biological function of this Mφ product. The fibroblast stimulation is assumed to play an active role in wound healing, which is of a great importance in the cornea. It is also noteworthy that recent studies (e.g., Grabner et al, 1982) have shown that IL 1-like molecules are produced by corneal epithelial cells, thus indicating that IL 1 molecules have a surprisingly wide spectrum of hormonal functions.

The findings concerning the effects of CsA on IL 1-mediated responses add useful information on the mode of action of this unique immunosuppressive drug. Studies by other investigators have indicated the selectivity in the effect of CsA and our results should help in defining the properties of the "resistant" and "sensitive" lymphocyte populations. The recent introduction of CsA as a therapeutic agent for certain ocular conditions further underlines the importance of the investigation on this compound.

Proposed Course: A major effort will be made to analyze further the mode of action of CsA, particularly with regard to its suggested capacity to interfere with interleukin 1-mediated responses. In addition, the effect of CsA on the release and biological functions of lymphocyte-made mediators (mainly interleukin 2 and mediators of inflammation) will be examined.

NEI Research Program: Retinal and Choroidal Diseases--Inflammatory Disorders; Corneal Diseases--Corneal Transplantation and Wound Healing; Cataract--Cataract Induced by Environmental and Toxic Effects

Publications:

Gery I, Zigler JS Jr, Brady RO, Barranger JA: Selective effects of glucocerebroside (Gaucher's storage material) on macrophage cultures. J Clin Invest 68:1182-1189, 1981.

Gery I, Seminara D, Derr J, Barranger JA: Production and release of lymphocyte activity factor (interleukin 1) by human monocytes and their derived macrophages, in Resch K, Kirchner H (eds): Mechanisms of Lymphocyte Activation. Amsterdam, Elsevier/North Holland Biomedical Press, 1981, pp 541-543.

Gery I, Davies P, Derr J, Krett N, Barranger JA: Relationship between production and release of lymphocyte activating factor (interleukin 1) by macrophages. I. Effects of various agents. Cell Immunol 64:293-303, 1981.

Oppenheim JJ, Gery I: Interleukin 1 is more than an interleukin. Immunol Today 3:113-119, 1982.

Gery I: Production and assay of interleukin-1 (IL-1), in Fathman CG, Fitch FW (eds): Isolation, Characterization and Utilization of T Lymphocyte Clones. New York, Academic Press (in press).

Mochizuki M, Zigler JS Jr, Russell P, Gery I: Lens epithelial cell cultures: a system for investigating lenticular damaging processes. Invest Ophthalmol Vis Sci 22(suppl):150, 1982.

Zigler JS Jr, Gery I, Kinoshita JH: Effects of lipid peroxidation products on the cultured lens. Invest Ophthalmol Vis Sci 22(suppl):151, 1982.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00069-05 LVR

PERIOD COVERED

October 1, 1981, to September 30, 1982
TITLE OF PROJECT (80 characters or less)

Immune Responses to Ocular Antigens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Igal Gery	Ph.D.	Chief, Section on Experimental Immunology	LVR	NEI
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	Manabu Mochizuki	M.D.	Visiting Associate	LVR	NEI
	W. Gerald Robison, Jr.	Ph.D.	Chief, Section on Experimental Anatomy	LVR	NEI
	Julia Derr	B.S.	Biologist	LVR	NEI

COOPERATING UNITS (if any)

- 1) Dept. of Medicine, Northwestern University Med. School, Chicago, IL
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LAB/BRANCH

Laboratory of Vision Research

SECTION

Section on Experimental Immunology

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

PROFESSIONAL:

OTHER:

1.5

1.3

0.2

CHECK APPROPRIATE BOX(ES)

 (a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER (a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Mechanisms involved in the development of pathogenic autoimmune processes in the eye have been studied by using an animal disease, experimental autoimmune uveitis (EAU). This disease is considered a possible model for certain human ocular diseases. Findings of specific interest are: (1) transfer of EAU to naive rats was achieved with highest efficacy when lymph node cells from donors with EAU were used, following preincubation with the sensitizing S-antigen. (2) The transferred EAU was prevented by treating the recipient rats with the immunosuppressive drug, cyclosporin A. This finding indicates that recruitment and clone expansion are essential for the development of EAU. (3) Studies with rats of inbred strains with different genetic makeup were extended, and confirmed our preliminary observation that genetic factors affect the susceptibility of rats to EAU. In addition, it was found that the development of disease in relatively resistant strains of rats was significantly enhanced by treatment with Bordetella pertussis bacteria, thus indicating the important role of environmental factors in determining an individual's susceptibility to EAU.

Project Description:

Objectives: Our studies of the development of experimental autoimmune uveitis (EAU) in rats have been extended, aimed in particular at the following aspects: (1) the participation of lymphocytes and their products in the pathogenesis of EAU, the cellular processes which take place during the development of the disease, and possible means of manipulating them. (2) The role of genetic and environmental factors in regulating the susceptibility of animals to induction of the disease.

Methods Employed: Rats of inbred strains were supplied mainly by commercial sources or the NIH breeding facilities. The AVN rats were descendants of a breeding pair kindly provided by Dr. David Gasser, University of Pennsylvania; the genetically defined backcrossed hybrids were developed and supplied by Dr. R. Michael Williams, Northwestern University. EAU was induced by immunization with the retinal S-antigen emulsified in complete Freund's adjuvant. In certain experiments, immunized rats were injected with killed Bordetella pertussis bacteria, 10 billion per rat, given intravenously. Transfer of EAU to naive recipients was carried out by injecting them with lymphocytes from draining lymph nodes or spleens of rats with EAU. The injected cells were usually preincubated for 3 days with S-antigen or concanavalin A. Treatment with cyclosporin A (CsA) consisted of seven daily intramuscular injections, 10 mg/kg each, starting one day before the cell transfer.

Major Findings: Lymph node cells from S-antigen-immunized rats were found to be highly efficient in transferring EAU following their preincubation with the S-antigen: as few as 5 million such cells were capable of transferring severe EAU to naive recipients. The lymph node cells were clearly superior in this function to similarly treated spleen cells from the same donors. The efficacy of lymph node cells in transferring the immune capacity was further underlined by the finding that rats receiving these cells had intense skin reactions to S-antigen of both the Arthus (antibody-mediated) and delayed (cellular mediated) types. In addition, recipients of the S-antigen-stimulated lymph node cells produced high levels of specific antibodies to S-antigen. In contrast, recipients of spleen cells cultured with Con A had only delayed type response to S-antigen, and no specific antibodies to this antigen were detected in their sera.

The system of EAU transfer was further analyzed by using the immunosuppressive drug, cyclosporin A (CsA). Treatment with CsA abolished the development of EAU in recipients of S-antigen-stimulated lymph node cells. CsA is known to affect mostly the clone expansion and recruitment of T lymphocytes; thus, this finding suggests that these processes play a major role in the transfer of EAU.

The studies concerning the effect of the genetic makeup on the development of EAU have produced the following new findings: (1) backcrossed hybrid rats of Lewis and BN ancestors which were typed for the RT1 locus developed EAU in a pattern indicating that different genetic factors control the susceptibility to EAU and another autoimmune disease, experimental allergic encephalomyelitis (EAE). Thus, 2/4 rats of the RT type of n/n developed EAU, in contrast to their known complete resistance to EAE. In addition, only 4/7 rats of the RT type of 1/n developed EAU, while EAE was

found to develop in actually all of these animals. (2) The role of environmental factors in the development of EAU was indicated by experiments with another rat inbred strain, AVN. These rats, which are completely resistant to EAE, developed EAU in about 20% of cases. However, all AVN rats developed severe EAU after being injected with the B. pertussis bacilli.

Significance to Biomedical Research and the Program of the Institute: The findings showing the high efficacy of lymph node cells in transferring EAU further establish the assumption that specifically sensitized lymphocytes play a major role in the pathogenesis of this disease. However, the finding that humoral immune response was also well developed in the recipients of the lymph node cells suggests that the humoral limb of the response to S-antigen participates as well in the pathogenic process. It is noteworthy that the involvement of immediate hypersensitivity in EAU is strongly indicated by recent data of Faure's group (1981) and by our finding concerning the effect of B. pertussis on EAU development (see below).

As mentioned above, the finding that treatment with CsA inhibits the development of EAU in recipients of lymph node cells suggests that recruitment and/or clone expansion are crucial processes for induction of this passive disease. Moreover, since the transferred lymphocytes are assumed to be presensitized, the data are interpreted to indicate that clone expansion and recruitment are processes which take place in relatively late stages of the pathogenic autoimmune process. The latter assumption thus supports the possible usefulness of drugs like CsA during flare-ups or late phases of human diseases of autoimmune origin and is in line with the beneficial effect of CsA in the preliminary study with human patients, as reported in this volume by Dr. R.B. Nussenblatt of the NEI Clinical Branch.

The findings with the genetically defined rats further establish the differences in the genetic regulation of the development of EAU and EAE. Of particular interest are our findings with the AVN rats, that the incidence of EAU increased strikingly by treating the animals with B. pertussis. These bacteria are known to enhance the immediate hypersensitivity response; thus, our finding supports the notion that this type of response may also affect the pathogenic mechanism of EAU, perhaps by affecting the retinal blood barrier.

Proposed Course: The system of EAU transfer by cultured lymphocytes will be further utilized in order to understand better the mechanisms by which lymphoid cells become exceedingly effective in causing EAU. In addition, attempts will be made to ascertain whether the active cells are a part of a certain lymphocyte subset.

The possible genetic regulation of EAU will be further examined by including in the study a new series of genetically defined rats. These lines of animals were recently developed by Dr. R.M. Williams (Northwestern University), and are unique in their different genetic makeup.

The possible involvement of immediate hypersensitivity in EAU development will be examined by using drugs which selectively inhibit the production and release of mast cell mediators. These drugs were successfully employed in inhibiting the induction of EAE in experimental mice (S. Linthicum, 1982). Both actively and passively induced EAU are to be tested with these drugs.

NEI Research Program: Retinal and Choroidal Diseases--Inflammatory Disorders

Publications:

Nussenblatt RB, Shichi H, Kuwabara T, Cevario S, Gery I: Resemblance between rhodopsin kinase and S-antigen induced uveitis. Brit J Ophthalmol 65:778-782, 1981.

Nussenblatt, RB, Gery I, Kuwabara T, deMonasterio F, Wacker WB: The role of the retinal S-antigen in primate uveitis, in Helmsen RJ, Suran AA, Gery I and Nussenblatt RB (eds): Immunology of the Eye, Workshop II. Washington, Information Retrieval Inc. 1981, pp 49-65.

Nussenblatt RB, Rodriques MM, Salinas-Carmona MC, Gery I, Cevario S, Wacker WB: Modulation of experimental autoimmune uveitis with cyclosporin A. Arch. Ophthalmol 100:1146-1149, 1982.

Salinas-Carmona, MC, Nussenblatt, RB, Gery I: Experimental autoimmune uveitis in athymic nude rat. Europ J Immunol (in press).

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Nussenblatt RB, Salinas-Carmona MC, Waksman BH, Gery I: Cyclosporin A: Alterations of the cellular immune response in S-antigen induced experimental autoimmune uveitis. Int. Arch. Allergy (in press).

Gery, I, Nussenblatt RC: Immunosuppressive drugs and therapy in ophthalmology, in Seara ML (ed): Handbook of Experimental Pharmacology, Volume on Pharmacology of the Eye Berlin, Springer-Verlag (in press).

Gery I, Shichi H, Robison WG, Jr, El-Saied M, Nussenblatt RB: Transfer of experimental autoimmune uveitis (EAU) by cultured lymphocytes. Invest Ophthal Visual Sci 22(suppl):213, 1982.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00129-10 LVR

PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Anatomical and Pathological Studies of Ocular Tissues

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Toichiro Kuwabara	M.D.	Chief, Section on Experimental Pathology	LVR NEI
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	Yoshio Akagi	M.D.	Visiting Scientist	LVR NEI
	Douglas Sisk	M.S.	Predoctoral Fellow	LVR NEI

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Vision Research

SECTION

Section on Experimental Pathology

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.1	2.1	1.0

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Histopathological studies were conducted on numerous human and animal eyes by transmission and scanning electron microscopy, as well as by histochemistry and histology. In studies designed to determine the etiology of gyrate atrophy, intravitreal injection of L-ornithine hydrochloride in physiologic saline solution caused marked edema specifically in the pigment epithelium of Sprague-Dawley strain albino and Evans black hooded rats and rhesus and cynomologus monkeys. Swelling of the pigment epithelial cells, which was most prominent four hours after the injection, disappeared by 24 hours. However, many pigment epithelial cells gradually degenerated resulting in patches of denuded areas. The photoreceptor cells overlying the damaged pigment epithelium degenerated secondarily.

Project Description:

Objectives: Clarify the normal structure and function of eye cells in order to add to an understanding of the pathophysiology of various eye diseases. Also, to study systematically the eye with naturally occurring diseases to elucidate further the pathogenesis involved.

Methods Employed: A large number of clinicopathological specimens sent to this laboratory from various eye research centers throughout the world were studied. Details on individual experiments on animals are described under Major Findings.

These eye tissues were fixed in glutaraldehyde solution and processed for transmission and scanning electron microscopy. Depending on specific diseases, various types of histochemical reactions were applied on cryo-, frozen, paraffin and plastic sections.

Major Findings: In the present experiments, injection of a small amount of ornithine into the vitreous caused selective damage to the pigment epithelium. Intravitreal injection of various doses of arginine, a basic amino acid precursor of ornithine, did not cause any damage in the pigment epithelium. Also, many other amino acids were non-toxic to the pigment epithelium, although exogenous administration of various amino acids has been known to cause cellular damage in the retina. Marked swelling, which led to subsequent degeneration, seemed to be the first morphologic reaction of the pigment epithelium to the excess amount of the exogenous ornithine. Since ornithine- δ -aminotransferase is presumably absent in the pigment epithelial cells of gyrate atrophy patients, these cells may be more vulnerable to elevated plasma levels of ornithine. The present animal model supports the hypothesis that the initial lesion in gyrate atrophy occurs in the pigment epithelium. Degeneration and atrophy of the photoreceptor cells, which occur progressively in the disease, may be secondary to the primary damage in the pigment epithelial cell. The associated choroidal atrophy seen clinically may also represent secondary changes to degeneration and atrophy of the pigment epithelium. These secondary changes have been noted in this experimental model.

The present intravitreal injection technique did not cause any inflammatory reaction. The administration of small amounts of experimental agents into the vitreous seems to be an appropriate method to study pharmacologic effects on the retina.

Significance to Biomedical Research and the Program of the Institute: The staff of this section is able to pursue a multidisciplinary study to attack problems which are directly related to clinical ophthalmology. Further clarification of the normal and abnormal structure and function of ocular tissues and cells is a significant part of eye research.

Proposed Course: Similar projects are actively ongoing and will be continued in the next fiscal year.

NEI Research Program: Retinal and Choroidal Diseases--Developmental and Hereditary Disorders/Inflammatory Disorders; Corneal Diseases--Corneal Edema, Endothelial Dysfunction Dystrophies, and Inherited Disorders/Corneal Transplantation and Wound Healing; Cataract--The Normal Lens/Cataract Induced by Environmental and Toxic Effects, Glaucoma--Primary Open-Angle Glaucoma, Etiology, Epidemiology, Management, and Therapy/Secondary Glaucomas

Publications:

Russell P, Uga S, Zigler S, Kaiser-Kupfer M, Kuwabara T: Studies using human lenses from a family displaying hereditary congenital cataracts. Vis Res 21:169-172, 1981.

Kirchoff H, Kuwabara T, Barile MR: Pathogenicity of Spiroplasma sp. strain SMCA in Syrian hamsters: clinical, microbiological and histological studies. Infect and Immunity 31:445-452, 1981.

Kuwabara T, Ishikawa Y, Kaiser-Kupfer MI: Experimental model of gyrate atrophy in animals. Ophthalmol 88:331-334, 1981.

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Sisk DR, Kuwabara T, Walk RD, Kirsch AD, Ishikawa, Y: Visual behavior in albino rats after glutamate-induced retinal degeneration. Invest Ophthalmol Vis Sci 22(suppl):249, 1982.

Carter-Dawson L, Kuwabara T, Bieri JG: Intrinsic, light-independent, regional differences in photoreceptor-cell degeneration in vitamin A-deficient rat retinas. Invest Ophthalmol Vis Sci 22:249-252, 1982.

Yajima Y, Akagi Y, Kador P, Kuwabara T: Immunohistochemical demonstration of aldose reductase in the human eye. Invest Ophthalmol Vis Sci 22(suppl):286, 1982.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
**NOTICE OF
INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 EY 00003-10 LVR

PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Cataracts

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Deborah A. Carper	B.A.	Biologist	LVR	NEI
	Roland Herrmann	M.D.	Visiting Fellow	LVR	NEI
	Yoshio Akagi	M.D.	Visiting Scientist	LVR	NEI

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Vision Research

SECTION

Section on Lens and Cataract

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

3.3

PROFESSIONAL:

2.3

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS

(b) HUMAN TISSUES

(c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Current investigations are being conducted on the events leading to the formation of several types of cataracts. Diabetic or sugar cataract formation initiated by the enzyme aldose reductase is being studied. Methods for controlling the onset of these cataracts through the regulation of this enzyme are being developed. Hereditary cataract formation is also being studied in a strain of mice developed in our laboratory. Known as the Philly mouse, these mice develop osmotic cataracts by an as yet unknown mechanism.

Project Description:

Objectives: To study the mechanism of cataract formation and to develop methods for its regulation.

Methods Employed: Sugar cataract formation can be induced in animals by either the use of a galactose enriched diet or through the use of appropriate chemical agents such as streptozotocin. Biochemical methods used for the purification of the enzyme include column chromatography, affinity chromatography, polyacrylamide gel electrophoresis (PAGE) and high pressure liquid chromatography (HPLC). Immunohistochemical analyses include the use of Ouchterloney plates and Laurell immunoelectrophoresis. Computational methods for enzyme analysis, inhibitor structure-activity studies and corneal reepithelialization studies require the use of the PROPHET and DCRT computer systems.

Major Findings: Rat lens aldose reductase (RLAR) was purified using a modified procedure of our previously developed method for the rapid purification of human placental aldose reductase (HPAR). This consisted of ammonium sulfate fractionation (50-70%) followed by affinity chromatography with Amicon Matrix Gel Orange A. The RLAR which appears to be closely associated with α and β -crystallins has a higher affinity for the dye matrix column than human placental aldose reductase. The purified enzyme obtained upon elution from the column appears as a closely spaced doublet of approximately 38K MW on SDS-PAGE which does not cross-react with antibodies raised against the single 38K MW HPAR. Antibodies raised against the two RLAR bands, however, do form a line of partial identity with HPAR. Differences in the susceptibility of rat lens, human lens and human placental aldose reductase to inhibition have also been observed suggesting that inhibitors designed for clinical use must eventually be evaluated with human enzyme. Currently "no universal" inhibitor exists.

Although immunohistochemical and inhibition differences have been observed between RLAR and HPAR, kinetic studies indicated that both RLAR and HPAR have similar substrate affinities. Both enzymes display greater affinity for aliphatic and aromatic aldehydes than for aldose sugars. Compared to DL-glyceraldehyde, RLAR displayed an 80 fold greater affinity for p-nitrobenzaldehyde (dissolved in 5% DMSO) and a 1000 fold decreased affinity for D-glucose. Both enzymes displayed only trace activity with 200 mM D-gulonic acid.

In studies of aldose reductase from either rat lens or human placenta with various classes of chemical inhibitors, we have observed the existence of a stereospecific inhibitor site independent of either the substrate or nucleotide cofactor fold at which inhibitors can reversibly react through a 'charge transfer' mechanism. This site can be irreversibly alkylated with 2'-bromo-4-nitroacetophenone. Using computer molecular modeling and molecular orbital studies of a variety of aldose reductase inhibitors, the minimum structural requirements of the inhibitor site has been estimated. The site includes two coplanar lipophilic regions joined by a nonplanar "charge transfer" pocket which contains an acidic and basic residue which can reversibly react with one another through an available carbonyl group present

on the inhibitors.

The Philly mouse is a derivative of the Swiss-Webster strain developed in our laboratory. This mouse develops an osmotic cataract during the fourth postnatal week. The Philly cataract progresses from an initial faint subcapsular opacity to a dense nuclear cataract in about one month. Crystallin synthesis is severely depressed in the fiber cells of the Philly cataract. This appears to be caused, at least in part, by ionic changes within the lens which interfere with the translation of crystallin messenger RNAs. In addition to the general reduction in crystallin synthesis, a β -crystallin polypeptide with a molecular weight near 27,000 (27 K) is selectively missing from the Philly cataract.

Cell-free translation tests showed that the messenger RNA (mRNA) for a major β -crystallin polypeptide with a molecular weight near 27,000 (27 K) in the Swiss-Webster mouse lens appears during the second postnatal week. A functional mRNA encoding the 27 K β -crystallin polypeptide fails to appear in the homozygous, hereditary, Philly mouse cataract. Breeding experiments indicated that the Philly cataract is a dominantly inherited trait and that accumulation of the 27K β -crystallin polypeptide is a codominant characteristic. Thus, the heterozygous Philly lens has intermediate levels of the 27K β -crystallin polypeptide and, interestingly, exhibits delayed onset of the cataract. The absence of functional 27K β -crystallin mRNA is the earliest lesion reported yet for the Philly lens and points to a transcriptional or posttranscriptional developmental defect in this hereditary cataract.

Significance to Biomedical Research and the Program of the Institute: Worldwide, cataract is one of the major causes of blindness while vision loss due to cataract formation prior to surgery in the United States presents a major public health problem. The diabetic population is especially prone to cataract formation and other ocular complications including retinopathy and corneal reepithelialization. Through the study of sugar cataracts and aldose reductase regulation, methods for the control of diabetic cataract formation and possibly the control of other diabetic complications can be developed.

Proposed Course: These studies will be continued. The biochemical properties of purified aldose reductase from human placenta and rat lens will be further compared. The mechanism of action of aldose reductase inhibitors will be studied in detail along with the structure activity relationships of the inhibitors. Through such studies the minimum requirements of the inhibitory site may be determined so that more active inhibitors may be designed.

NEI Research Program: Cataract--Diabetic and Metabolic Cataract

Publications:

Kador PF, Sharpless NE, Goosey JD: Aldose reductase inhibition by anti-allergy drugs, in Weiner H, Wermuth B (eds): Enzymology of Carbonyl Metabolism: Aldehyde Dehydrogenase and Carbonyl Reductase. New York, Alan R. Liss, Inc., (in press).

Datiles MB, Kador PF, Fukui HN, Hu TS, Kinoshita JH: Corneal re-epithelialization in galactosemic rats. Invest Ophthalmol Vis Sci (in press).

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Carper D, Shinohara T, Piatigorsky J, Kinoshita JH: Deficiency of functional messenger RNA for a developmentally regulated β -crystallin polypeptide in a hereditary cataract. Science 217:463, 1982.

Datiles M, Hu T-S, Kador P, Robison WG Jr, Kinoshita J: Diabetic rat corneas: glucose levels related to recovery from scraping. Invest Ophthalmol Vis Sci 22(suppl):25, 1982.

Kador PF, Sharpless NE: A proposed model of the aldose reductase inhibitor site. Invest Ophthalmol Vis Sci 22(suppl):33, 1982.

Herrmann RK, Kador PF, Kinoshita JH: Properties of purified rat lens aldose reductase. Invest Ophthalmol Vis Sci 22(suppl):156, 1982.

Yajima Y, Akagi Y, Kador P, Kuwabara TK: Immunohistochemical demonstration of aldose reductase in the human eye. Invest Ophthalmol Vis Sci 22(suppl):156, 1982.

Carper D, Shinohara T, Piatigorsky J, Kinoshita JH: A missing β -crystallin in the Philly mouse cataract. Invest Ophthalmol Vis Sci 22 (suppl):158, 1982.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U. S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 EY 00136-10 LVR																									
PERIOD COVERED October 1, 1981, to September 30, 1982																													
TITLE OF PROJECT (80 characters or less) Chemistry and Metabolism of the Lens																													
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																													
<table> <tr> <td>PI:</td> <td>Paul Russell</td> <td>Ph.D.</td> <td>Research Chemist</td> <td>LVR</td> <td>NEI</td> </tr> <tr> <td>Other:</td> <td>Tien-sheng Hu</td> <td>M.D.</td> <td>Visiting Scientist</td> <td>LVR</td> <td>NEI</td> </tr> <tr> <td></td> <td>Lorenzo Merola</td> <td>M.S.</td> <td>Chemist</td> <td>LVR</td> <td>NEI</td> </tr> <tr> <td></td> <td>Geeta Pararajasegaram</td> <td>B.S.</td> <td>Guest Worker</td> <td>LVR</td> <td>NEI</td> </tr> </table>						PI:	Paul Russell	Ph.D.	Research Chemist	LVR	NEI	Other:	Tien-sheng Hu	M.D.	Visiting Scientist	LVR	NEI		Lorenzo Merola	M.S.	Chemist	LVR	NEI		Geeta Pararajasegaram	B.S.	Guest Worker	LVR	NEI
PI:	Paul Russell	Ph.D.	Research Chemist	LVR	NEI																								
Other:	Tien-sheng Hu	M.D.	Visiting Scientist	LVR	NEI																								
	Lorenzo Merola	M.S.	Chemist	LVR	NEI																								
	Geeta Pararajasegaram	B.S.	Guest Worker	LVR	NEI																								
COOPERATING UNITS (if any)																													
LAB/BRANCH Laboratory of Vision Research																													
SECTION Section on Lens and Cataract																													
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205																													
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:																											
4.0	3.0	1.0																											
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<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																													
SUMMARY OF WORK (200 words or less - underline keywords)																													
<p>Investigation into the alterations in <u>protein composition</u> of the <u>lens</u> were conducted with particular reference to <u>membrane proteins</u>. Development and refinement of <u>in vitro model systems</u> for <u>cataract development</u> have continued. Results from these <u>in vitro</u> systems indicate many of the steps during cataract development proceed in a very similar manner regardless of the <u>initiating event in cataractogenesis</u>. <u>Glyceraldehyde phosphate dehydrogenase</u>, an enzyme in the <u>glycolytic pathway</u>, has been studied <u>in vivo</u> and <u>in vitro</u>. The possible <u>inactivation</u> of the enzyme during cataract formation has been under investigation. Cultures of <u>lens epithelial cells</u> as well as other cells from <u>tissue culture</u> have been utilized to study <u>basement membrane formation</u>, <u>cytotoxic agents</u>, and enzymes such as <u>aldose reductase</u>. These cells are beneficial because they facilitate the study of biological events in a well-defined and easily manipulated system.</p>																													

Project Description:

Objectives: The purpose of the work is to obtain understanding of the events which follow insults to the lens. Our objective is to determine how events like protein alteration proceed during cataract development. The work has concentrated on membrane and membrane protein changes since these appear to be relatively early alterations which lead inevitably to cataract formation. Use of cultured cells from the lens aids in the understanding of the way in which the epithelial cells of the lens react to altered growth environments.

Methods Employed: Fresh lenses and lenses incubated *in vitro* have been used. Proteins and enzymes extracted from these lenses have been studied using spectroscopic, gel electrophoretic, and immunochemical methods. Electron microscopic methods have also been employed with the whole lenses.

Major Findings: Cataract development in the lens results in many changes in the proteins as well as alteration in enzyme activities. One aspect of this problem which is particularly interesting is the change in membrane protein during cataractogenesis. The main intrinsic membrane polypeptide (MIP) in the lens fiber cells has a molecular weight of 26,000. This protein is associated with the gap junctions of the mature fiber cells. During cataract formation in the Nakano and Philly mouse strains, there is an increase in a 23,000 molecular weight (MW) component and a decrease in the MIP. This decrease in MIP correlates with the decrease in gap junctions in these lenses. Other laboratories have shown for bovine lenses that a cleavage of the MIP into a 23,000 MW component is possible when a proteolytic enzyme is added to the MIP. Thus, the post translational modification of the MIP which was observed with the Nakano and Philly cataractous strains of mice may be some type of proteolytic cleavage to yield a 23,000 MW component.

Because of the animal-to-animal variation in the colonies of mice, an *in vitro* system for studying this post translational modification was developed. *In vitro* incubation of normal mouse lenses in glucose-deprived medium or medium containing a calcium ionophore resulted in the alteration of MIP to the 23,000 MW component. Changes in the MIP can now be studied using this *in vitro* system which is both rapid and reproducible. Lenses from mice and rats can be used with this incubation procedure.

By electron microscopy, the lenses after incubation in the glucose deprived medium resemble lenses obtained from galactose fed animals. Feeding a 50% galactose diet to young rats results in cataract formation. The bow region of these cataractous lenses is morphologically destroyed and the membranes between cells in the anterior cortex become very thin in appearance and lose gap junctions. The *in vitro* incubation results in a lens morphologically similar to that obtained during this *in vivo* cataract formation.

Another change which can be observed with the *in vitro* system is a loss of some enzyme activities. One particularly interesting enzyme, glyceraldehyde phosphate dehydrogenase, is rapidly inactivated during the course of the incubation period. This enzyme is an important one in the glycolytic pathway. In the red blood cells, as much as 60% of this enzyme is closely associated with the membrane under normal conditions. When this enzyme is associated with

the membrane, it is inactive. Association of glyceraldehyde phosphate dehydrogenase to the lens membrane can also be shown in the normal lens. It appears that about 40% is associated with the bovine fiber cells membrane, although the amount of enzyme associating with the lens membrane varies from species to species. This enzyme has a very reactive sulfhydryl group at the active site which can be readily oxidized to inactivate the enzyme irreversibly. Work by other investigators shows that oxidation of sulfhydryl groups occurs in the vicinity of the membrane during cataract formation. This oxidation may be responsible for the loss in enzyme activity that is seen with in vitro incubation. The glyceraldehyde phosphate dehydrogenase from human and bovine lenses is similar. The enzyme has a molecular weight of about 140,000 and has an isoelectric point above pH 7.5. The enzyme from rat and mouse lenses has a slightly lower molecular weight, and the oxidation of the sulfhydryl is extremely rapid. The enzyme from mouse lens is extremely labile making it difficult to study.

In addition to whole lenses or lenses incubated in vitro, cultured mouse lens epithelia cells have also been studied. In collaboration with other workers, we have used these cells to study the production of basement membrane by the lens as well as the influence of retinal derived growth factors on cell mitosis. Another factor in lens metabolism, the enzyme aldose reductase, has been studied with these cells as well as those derived from a retinoblastoma cell line. The enzyme is present in cultured cells, and addition to the culture of sugars such as galactose and xylose result in the formation of sugar alcohols. Use of cultured cells will aid in determining the effects that sugar alcohols have in on cellular metabolism.

Significance to Biomedical Research and the Program of the Institute:
Investigation of the steps involved in cataract formation is essential to the understanding of cell metabolism and integrity after an insult to the lens. Certainly the maintenance of cellular membrane and gap junction communication is important to lens clarity. A reproducible method of examining the steps in the formation of cataract such as the in vitro incubation of lens is important to this understanding. The use of tissue culture is one method for examining the influence of environmental alterations on lens cell metabolism.

Proposed Course: Investigations will continue on alterations of the fiber cell membrane during cataract formation. Particular emphasis will be given to the mechanism by which MIP is altered in cataractogenesis. In addition, studies using cultured cells will concentrate on differences in metabolism in relation to conditions which influence cataract formation such as osmotic change and changes in electrolytes.

NEI Research Program: Cataract--The Normal Lens

Publications:

Russell P: Changes in intrinsic membrane proteins in mouse lens during cataractogenesis, in Sears M (ed): New Directions in Ophthalmic Research. New Haven, Yale University Press, 1981, pp 109-124.

Project No. Z01 EY 00136-10 LVR

Rohrbach, DH, Russell P, Church, RL: In vitro production of basement membrane components by a clonal line of mouse lens epithelial cells. Current Eye Res 5:267-273, 1981.

Roy D, Garner MH, Spector A, Carper D, Russell P: Investigation of Nakano lens proteins. Exp Eye Res 34:909-920, 1982.

Salinas MC, Russell P, Hooks J, Gery I, Nussenblatt R: Mitogen induced suppressor factors from human lymphocytes II Biological and physiochemical properties. Cell Immunity (in press).

Hu T-S, Russell P, Kinoshita JH: In vitro incubation paralleling changes occurring during mouse cataract formation. Exp Eye Res (in press).

Russell P, Merola L, Yajima Y, Kinoshita JH: Aldose reductase activity in a cultured human retinal cell lens. Exp Eye Res (in press).

Mochizuki M, Zigler JS Jr, Russell P, Gery I: Lens epithelial cell cultures: A system for investigating lenticular damaging processes. Invest Ophthalmol Vis Sci 22(suppl):150, 1982.

Russell P, Kinoshita JH: Properties of lens glyceraldehyde-3-phosphate dehydrogenase. Invest Ophthalmol Vis Sci 22(suppl):157, 1982.

Hu T-S, Russell P, Kinoshita JH: In vitro incubation paralleling changes occurring during mouse cataract formation. Invest Ophthalmol Vis Sci 22 (suppl):158, 1982.

Merola LO, Russell P, Yajima Y, Kinoshita JH: Aldose reductase in a cultured human retinal cell line. Invest Ophthalmol Vis Sci 22 (suppl):188, 1982.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (DO NOT use this space)		U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00105-03 LVR		
PERIOD COVERED October 1, 1981, to September 30, 1982					
TITLE OF PROJECT (80 characters or less) Structure and Composition of Lens Crystallins with Respect to Cataract Development					
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT					
PI:	J. Samuel Zigler, Jr.	Ph.D	Research Biologist	LVR	NEI
Other:	Richard Bodaness	M.D., Ph.D.	Senior Staff Fellow	LVR	NEI
	Tien-sheng Hu	M.D.	Guest Worker	LVR	NEI
	Igal Gery	Ph.D.	Visiting Scientist	LVR	NEI
COOPERATING UNITS (if any) Howard M. Jernigan, Jr., Univ. of Tennessee Center for Health Sciences					
LAB/BRANCH Laboratory of Vision Research					
SECTION Section on Lens and Cataract					
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205					
TOTAL MANYEARS:	1.9	PROFESSIONAL:	1.9	OTHER:	0
CHECK APPROPRIATE BOX(ES)					
<input type="checkbox"/> (a) HUMAN SUBJECTS		<input checked="" type="checkbox"/> (b) HUMAN TISSUES		<input type="checkbox"/> (c) NEITHER	
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS					
SUMMARY OF WORK (200 words or less - underline keywords) It is known that <u>oxidative stress</u> leads to a number of well-characterized structural modifications in <u>lens crystallins</u> . These modified lens proteins accumulate within the lens and may play a major role in formation of some <u>senile cataracts</u> . We have proposed that <u>singlet oxygen</u> produced photodynamically may account for the oxidative damage. We have shown that <u>photosensitizers</u> endogenous to the lens can support the process, as could photosensitizing drugs which reach the lens. We have recently been able to extend studies of possible oxidative mechanisms of cataractogenesis to cataracts associated with <u>chronic uveitis</u> and <u>retinal degenerative diseases</u> respectively. In the first case, the lens is exposed to <u>macrophages</u> which are known to produce active species of oxygen, particularly <u>hydrogen peroxide</u> and <u>superoxide</u> . Cultured lenses are damaged when exposed to activated macrophages, apparently by an oxidative mechanism. In the case of cataracts associated with retinal degeneration we believe that <u>peroxidation</u> of the <u>polyunsaturated lipids</u> from degenerating <u>rod outer segments</u> may generate toxic species which initiate cataract.					

Project Description:

Objectives: (1) To characterize better the structural modifications which accumulate in crystallins with age and to determine the relationship of these changes to cataractogenesis. (2) To determine the mechanisms responsible for the oxidative changes occurring in the lens. (3) To investigate specifically the etiology of particular classes of cataracts which may be initiated by oxidative mechanisms.

Methods Employed: Lens crystallins were isolated by gel chromatography and analyzed by standard electrophoretic and immunochemical techniques. Photochemical studies involved near ultraviolet or visible irradiation of protein solutions or intact lenses in the presence of various photosensitizing compounds. Lens culture experiments involved rat lenses cultured in modified TC-199 medium under standard culture conditions. Lens were stressed during culture by addition of specific drugs or other potentially toxic agents. Assessment of lens damage was by uptake of radioactive substances from the medium as measured by liquid scintillation counting.

Major Findings: Following up earlier studies involving photodynamic effects on lens crystallin solutions in which singlet oxygen-mediated modifications similar to those found in human lenses were produced, we have looked in depth at the same system utilizing intact lenses in organ culture. These studies have demonstrated that cultured lenses exposed to singlet oxygen generating systems are impaired not only in terms of membrane transport parameters, but also that the lens proteins undergo the same modifications which occur in solutions of isolated crystallins. These observations strengthen the hypothesis that oxidation via photodynamic action may contribute to senile nuclear cataractogenesis.

We found that products resulting from peroxidation of polyunsaturated lipids in degenerating rod outer segments may cause the posterior sub-capsular cataracts associated with retinal degenerative diseases. Rat lenses exposed in organ culture to ROS or the major polyunsaturated fatty acid of ROS undergo marked damage which correlates well with the degree of lipid peroxidation in the culture medium. Lens damage in the system is increased by addition of iron, which potentiates peroxidation, or is decreased by vitamin E, an inhibitor of lipid peroxidation. Studies in which semicarbazide was used to scavenge the aldehyde products of the peroxidation process showed that the toxic aldehydes (e.g. malondialdehyde) are responsible in large part for the damage incurred by the lenses in this system. Preliminary studies suggest that a similar mechanism could be responsible for the cataracts found in the RCS rat, a commonly studied model of hereditary retinal dystrophy.

Dr. Richard S. Bodaness has been conducting research on cellular defense mechanisms against singlet molecular oxygen, a species which may be involved in damage to both the lens and retina. His research has demonstrated that a constituent of all cells, NADPH (reduced nicotinamide adenine dinucleotide phosphate), in combination with cytoplasmic NADP-linked dehydrogenases such as isocitrate dehydrogenase, may provide a defense mechanism against singlet oxygen mediated toxicity to cytoplasmic constituents. Singlet oxygen is known to oxidize NADPH. The predominant oxidation product is enzymatically active

NADP. NADP can be reduced back to NADPH by NADP-linked dehydrogenases. Thus, a scavenging cycle may exist contributing to the protection of cytoplasmic components against singlet oxygen. Specifically, an enzyme, isocitrate dehydrogenase, was shown to be completely inactivated by singlet oxygen generated photochemically. When NADPH was present, the enzyme was partially protected and able to regenerate more reduced cofactor (NADPH) from NADP and thus contribute to its own preservation.

Significance to Biomedical Research and the Program of the Institute:
These studies are aimed at increasing knowledge of the relationship between oxidative stress in the eye and cataract. We believe that oxidation is a major factor in the formation of senile nuclear cataracts and also in the cataracts associated with chronic uveitis and retinal degeneration. Understanding the mechanism(s) at work in the etiology of these cataracts may make possible the development of means of slowing or preventing their development.

Proposed Course: Studies on the effects of singlet oxygen on the lens will continue, with emphasis both on the problems associated with the endogenous photosensitizers as well as those related to various therapeutic agents which have photosensitizing activity (e.g. chlorpromazine). Attempts will be made to analyze on the molecular level the changes produced in lens proteins so that the actual mechanisms of action of singlet oxygen in the lens can be determined.

The studies concerning the relationship between lipid peroxidation and cataract will continue with more emphasis on the RCS model system. Histological studies of RCS lenses are underway, and further assays will be undertaken to determine whether in fact lipid peroxidation is occurring in the retinal debris which accumulates in these eyes and, if so, whether toxic products diffuse through the vitreous such that they could initiate cataracts.

NEI Research Program: Cataract--Senile Cataract

Publications:

Zigler JS Jr, Carper DA, Kinoshita JH: Changes in lens crystallins during cataract development in the Philly mouse. Ophthalmic Res 13:237-251, 1981.

Gery I, Zigler JS Jr, Brody RO, Barranger JA: Selective effects of glucocerebroside (Gauchers storage material) on macrophage cultures. J Clin Invest 68:1182-1189, 1981.

Zigler JS Jr, Jernigan HM Jr, Perlmutter NS and Kinoshita JH: Photodynamic cross-linking of polypeptides in intact rat lens. Exp Eye Res (in press).

Zigler JS Jr, Gery I, Kessler D, Kinoshita JH: Macrophage mediated damage to rat lenses in culture: a possible model for uveitis-associated cataract. Invest Ophthalmol Vis Sci (in press).

Project No. Z01 EY 00105-03 LVR

Mochizuki M, Zigler JS Jr, Russell P, Gery I: Lens epithelial cell cultures: A system for investigating lenticular damaging processes. Invest Ophthalmol Vis Sci 22(suppl):150, 1982.

Zigler JS Jr, Gery I, Kinoshita JH: Effects of lipid peroxidation products on the cultured lens. Invest Ophthalmol Vis Sci 22(suppl):151, 1982.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00148-09 LVR		
PERIOD COVERED October 1, 1981, to September 30, 1982					
TITLE OF PROJECT (80 characters or less) Cyclic Nucleotides and Vision					
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT					
PI:	Gerald J. Chader Susan Gentleman	Ph.D. Ph.D.	Research Chemist Expert	LVR	NEI
Other:	R. T. Fletcher Robert L. Somers	M.S. B.S.	Chemist Chemist	LVR	NEI
COOPERATING UNITS (if any)					
1) Section on Ophthalmology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA					
2) Department of Anatomy, Erasmus University, Rotterdam, The Netherlands					
LAB/BRANCH Laboratory of Vision Research					
SECTION Section on Retinal and Corneal Metabolism					
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205					
TOTAL MANYEARS:	3.5	PROFESSIONAL:	1.5	OTHER:	2.0
CHECK APPROPRIATE BOX(ES)					
<input type="checkbox"/> (a) HUMAN SUBJECTS		<input checked="" type="checkbox"/> (b) HUMAN TISSUES		<input type="checkbox"/> (c) NEITHER	
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS					
SUMMARY OF WORK (200 words or less - underline keywords)					
<p>1) Distinct biochemical differences have been found between models for <u>early onset retinal degeneration</u> that were previously thought to belong to a single disease type. Although <u>cyclic nucleotide</u> metabolism is deranged in each animal model, quantitative and qualitative differences are observed in enzymes involved in regulating the concentration of both <u>cAMP</u> and <u>cGMP</u>. This indicates that there may be multiple etiologies of these diseases and that the causes of each disease must be separately established for each animal species or genetic variation.</p> <p>2) The role of cyclic nucleotides in visual cells other than the photoreceptor visual process appears to be complex having some similarities to well known brain fractions but also some distinct and potentially unique differences. <u>cAMP</u> in particular appears to be involved in <u>retinomotor movements in retina and pigment epithelium</u>.</p>					

Project Description:

Objectives: 1) To study the role of cGMP and cAMP and their enzymes of metabolism in normal retinas and those with retinal degeneration. 2) To study the general role of cyclic nucleotides in functioning of the retinal photo-receptor-pigment epithelial complex and also in the inner layers of the neural retina.

Methods Employed: Standard biochemical and neurochemical techniques are utilized.

Major Findings: 1) In several animal models for retinitis pigmentosa (C3H mouse, Irish setter and collie) retinal cGMP is abnormally high, reflecting a defect in outer segment (OS) phosphodiesterase (PDE) activity. In all these cases, increased cGMP and decreased PDE precedes morphological signs of the disease. In the setter, retinal calmodulin is also low although this is not the case in the collie. Differences in cyclic nucleotide metabolism are not observed in pigment epithelium (PE). In contrast, in the rds mouse (O20/A), OS fail to develop but both retinal cGMP and cGMP-PDE are low. Surprisingly, major differences are observed in PDE activities in PE-choroid. cAMP-PDE peaks at 30d in normal PE whereas in rds, it is high at 15-20d and drops well below normal by 30d. cGMP-PDE in normal PE peaks later than in rds and is 3-fold greater. Thus, not only are there biochemical differences in the retina between quite similar disease entities, but the rds mouse model indicates that the PE-choroid complex may be involved in the etiology of early onset retinal degeneration in some species.

2) Also under study is the coupling sequence between ligand binding at the neurotransmitter receptor, regulation of adenylyl cyclase activity and specific protein phosphorylation in vertebrate retina. Correlative studies of adenylyl cyclase modulation with ligand binding include dopamine, vasoactive intestinal peptide (VIP) and adenosine receptors. Binding assays for high affinity adenosine and low affinity dopamine sites have been developed. Binding assays for VIP receptors and low affinity adenosine receptors are in preparation. The developmental sequence of the appearance of these receptors in chick retina has been studied by autoradiography of specifically bound radiolabeled-ligand in frozen thin section. In these studies, we intend to correlate receptor binding with the regulation of adenylyl cyclase in membrane preparations from 7 to 18 day chick embryos.

Significance to Biomedical Research and the Program of the Institute: Only in thoroughly defining both normal and abnormal functioning of a tissue can one understand a disease process. It is hoped in this way ultimately to be able to slow down or even stop the degenerative process in the retinas of animal models of RP and finally in human retinitis pigmentosa itself.

Proposed Course: Experimentation will continue on control mechanisms in the normal retina and in genetic diseases of the retina.

NEI Research Program: Retinal and Choroidal Diseases--Developmental and Hereditary Disorders

Publications:

Chader GJ, Liu YP, Fletcher RT, Aguirre GA, Santos-Anderson R, T'so MOM: Cyclic GMP phosphodiesterase and calmodulin in early onset inherited retinal degenerations, in Miller W (ed): Current Topics in Membranes and Transport. New York, Academic Press, 1981, pp 133-156.

Chader GJ, Fletcher RT, Krishna G: Guanine nucleotides: Importance in the visual process of the rod outer segment, in Sears M (ed): New Directions in Ophthalmic Research. New Haven, Yale Univ Press, 1981, pp 191-206.

Woodford BJ, Liu YP, Fletcher RT, Chader GJ, Farber D, Santos-Anderson R, T'so MOM: Cyclic nucleotide metabolism in inherited retinopathy in collies: A biochemical and histochemical study. Exp Eye Res 34:703-714, 1982.

Burnside B, Evans M, Fletcher RT, Chader G: Induction of dark-adaptive retinomotor movement (cell elongation) in teleost retinal cones by cyclic adenosine 3'5'-monophosphate: J Gen Physiol 79:759-774, 1982.

Barbehenn EK, Noelker DM, Chader GJ, Passonneau JV: Effects of in vivo light adaptation on metabolites in frog retinal layers. Invest Ophthalmol Vis Sci 22(suppl):184, 1982.

Liu YP, Fletcher RT, Chader GJ, Sanyal S, Aguirre G: Cyclic nucleotides and morphology in inherited retinal degenerative rds mice (020/A). Invest Ophthalmol Vis Sci 22(suppl):249, 1982.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00146-01 LVR

PERIOD COVERED

April 5, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Cellular Proliferation in Diabetic Retinopathy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Leonard M. Hjelmeland	Ph.D.	Expert Consultant	LVR	NEI
Other:	J. Bressler	Ph.D.	Senior Staff Fellow	SN	NINCDS
	G. Grotendorst	Ph.D.	Staff Fellow	LDBA	NIDR
	A. Chrambach	Ph.D.	Research Chemist	RR	NICHD

COOPERATING UNITS (if any)

- 1) Reproductive Research Branch, NICHD
- 2) Surgical Neurology Branch, NINCDS
- 3) Laboratory of Developmental Biology and Anomalies, NIDR
- 4) Wilmer-Woods Institute, Johns Hopkins University Hospital

LAB/BRANCH

Laboratory of Vision Research

SECTION

Section on Retinal and Corneal Metabolism

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0.0

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Research is being conducted on the biological mechanisms which control proliferation of various cell types in diabetic retinopathy which include fibroblasts, glia, pigment epithelium, and vascular smooth muscle. Using wound repair as a general model, we have demonstrated that glial cells exhibit chemotaxis to platelet-derived growth factor (PDGF) in a fashion similar to that described in previous reports on smooth muscle and fibroblasts. Pigmented epithelial cells are currently being evaluated for chemotaxis to PDGF. As a consequence of the general wound repair model, each of these cell types is, in turn, being evaluated as a possible source of soluble factors which may be involved in neovascularization.

Project Description:

Objectives: To elucidate the sequence of cellular and biochemical events which lead to the development of neovascularization and fibroplasia in proliferative diabetic retinopathy. To isolate any soluble macromolecules which may mediate these events and develop methods to alter the course of the disease.

Methods Employed: Primary cell cultures of glial cells, fibroblasts pigmented epithelium, and vascular smooth muscle are established from chick, cow, monkey, and human eyes. The cells themselves are tested for chemotaxis to a variety of macromolecules thought to be involved in wound repair such as platelet derived growth factor. Such factors are in turn used to induce the synthesis of new proteins by the same cells in monolayer cultures. Serum free conditioned media are collected 24 hours after induction and fractionated by high performance liquid chromatography and preparative isoelectric focusing. The resulting fractions are tested for angiogenesis in the rabbit corneal pouch assay and the endothelial cell chemotaxis assay.

Major Findings: (1) Glial cells from the postnatal rat brain and 14 day chick retina have been shown to chemotax towards gradients of platelet-derived growth factor. This finding establishes both the role of PDGF and wound repair in gliosis, and at the same time demonstrates the fact that non-mesodermal cells can participate in the wound repair response, at least in the central nervous system. (2) Conditioned media from transformed human glial cells and possibly retinoblastoma contain chemoattractants for endothelial cells. In order to understand angiogenesis in the central nervous system, it is important to compare, for example, cortex with retina. Since attractants for endothelial cells have been isolated from bovine retina, we are comparing such material with attractants produced by human gliomas and retinoblastoma. These investigations should lead to large scale isolations.

Significance to Biomedical Research and the Program of the Institute: Establishing the sequence of cellular and biochemical events which lead to proliferative retinopathy could lead to the rational design of procedures or drugs which could modify the course of this disease.

Proposed Course: Smooth muscle cells, glial cells, fibroblasts, and pigment epithelium will be obtained in primary culture. Conditioned media from these cells will be examined for factors which are either angiogenic or chemotactic for endothelial cell. Such factors will be purified to the extent possible and characterized.

NEI Research Program: Retinal and Choroidal Diseases--Diabetic Retinopathy--Sickle Cell Retinopathy, and other Vascular Abnormalities

Publications:

Hjelmeland LM, Chrambach A: Formation of natural pH gradients in sequential moving boundary systems with solvent counterions (I): Theory. Electrophoresis (in press).

Buszas Z, Hjelmeland LM, Chrambach A: Formation of natural pH gradients in sequential moving boundary systems with solvent counterions (II): Predicted and experimental properties. Electrophoresis (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00124-02 LVR		
PERIOD COVERED October 1, 1981, to September 30, 1982					
TITLE OF PROJECT (80 characters or less) Metabolism of the Retina and Pigment Epithelium					
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT					
PI:	Shay-Whey M. Koh Gerald J. Chader	Ph.D. Ph.D.	Staff Fellow Research Chemist	LVR NEI LVR NEI	
Other:	A. Kyritsis	M.D.	Visiting Scientist	LVR NEI	
COOPERATING UNITS (if any)					
LAB/BRANCH Laboratory of Vision Research					
SECTION Section on Retinal and Corneal Metabolism					
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205					
TOTAL MANYEARS:	2.75	PROFESSIONAL:	2.75	OTHER:	0.0
CHECK APPROPRIATE BOX(ES)					
<input type="checkbox"/> (a) HUMAN SUBJECTS		<input type="checkbox"/> (b) HUMAN TISSUES		<input checked="" type="checkbox"/> (c) NEITHER	
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS					
SUMMARY OF WORK (200 words or less - underline keywords)					
<p>Characteristics of <u>metabolism</u> and function of the <u>neural retina</u> (NR) and <u>pigment epithelium</u> (PE) are studied in cultured cells and in freshly dissected tissue. Several new <u>neurotransmitter/neuromodulator</u> receptors have been identified in retina and pigment epithelial cells in culture. For example, PE cells respond to <u>VIP</u>, <u>prostaglandins</u>, <u>glucagon</u> and <u>β-adrenergic agonists</u>. Neural retinal cells respond to glucagon, VIP and <u>dopamine</u>. These substances may control important processes of NR and PE cells <i>in vivo</i>.</p>					

Project Description:

Objectives: To better understand the general metabolism of the retina-pigment epithelial unit and to apply this information to diseases which affect these tissues.

Methods Employed: PE and retinal cell cultures were maintained from tissues of the chick embryo. When appropriate, fresh tissues were dissected from the eye using a stereomicroscope. Biochemical analyses were performed using standard assay procedures as adapted in our laboratory for the particular ocular tissue.

Major Findings: Chick embryonic NR cells grow well in culture, exhibiting cells of both neuronal and glial character. When such cultures are treated with VIP, a 20-fold increase in cAMP is observed within 3 minutes. Similarly, glucagon elicits a 10-fold increase in cAMP, glucocorticoid hormone appears to modulate the VIP response. A striking dopamine response is also observed in newly plated cultures where neuronal-type cells are present. In older cultures, containing flat, glial-type cells and few cells of neuronal appearance, the dopamine response is lost. Several other effectors (e.g. TSH α -MSH, ACTH, histamine, adenosine) minimally affect cAMP in NR cells. Both VIP and glucagon also control cAMP levels in cultured human Y-79 retinoblastoma cells. Chick PE cells also differentiate well in culture. cAMP responses include: VIP (100-fold); glucagon (5-10-fold); PGE-1, (30-fold). PE cells also demonstrate a distinct β -adrenergic receptor with a 10-20-fold increase in cAMP seen with isoproterenol. The effect is blocked by propranolol and trifluoperazine. Binding of HYP, a β -antagonist, demonstrates a dissociation constant of 1.6 nMolar and about 300,000 binding sites/cell. Thus, cultured NR and PE cells respond to several transmitters/modulators, indicating that these substances may play important roles in retina and PE in vivo.

Significance to Biomedical Research and the Program of the Institute: The pigment epithelium is an important cell layer which acts as a partner with the retina in the visual process. Understanding the basic factors which promote differentiation and the basic factors which control PE cell metabolism should aid us in better understanding dysfunction of the PE-retina unit.

Proposed Course: The metabolic capabilities of the PE cell will be further investigated. Correlations with specific diseases of the PE-retina unit will be made.

NEI Research Program: Retinal and Choroidal Diseases--Retinal Pigment Epithelium

Publications:

Tamai M, Mizuno K, Chader G: In vitro studies on shedding and phagocytosis of rod outer segments in the rat retina: Effects of oxygen concentration. Invest Ophthalmol Vis Sci 22:439-448, 1982.

Chader GJ: Retinal biochemistry-a holistic view, in Clayton R, Reading W (eds): Problems of Normal and Genetically Abnormal Retinas. Edinburgh, Univ. Edinburgh Press (in press).

Chader GJ, Masterson E, Goldman A: Tissue culture of chick embryonic choroidal cells: Cell aggregation and pigment accumulation, in Clayton R, Truman D (eds): Stability and Switching in Cellular Differentiation. Edinburgh, Univ. Edinburgh Press (in press).

Chader GJ, Koh S-W, Masterson E: Effect of ornithine on macromolecular biosynthesis in embryonic pigment epithelium, in Clayton R, Reading W (eds): Problems of Normal and Genetically Abnormal Retinas. Univ. Edinburgh Press (in press).

Chader GJ, Koh S-W: Embryonic pigment epithelium in culture contains a distinct β -adrenergic receptor. Invest Ophthalmol Vis Sci 22(suppl):186, 1982.

Masterson E, Koh S-W, Chader G: Effect of ornithine on macromolecular biosynthesis in embryonic pigment epithelium. Invest Ophthalmol Vis Sci 22(suppl):250, 1982.

Koh S-W, Chader GJ: Specificity of phagocytosis in cultured pigment epithelial (PE) cells. Invest Ophthalmol Vis Sci 22(suppl):186, 1982.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00070-05 LVR

PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Vitamin A and Ocular Tissues

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Barbara Wiggert	Ph.D.	Research Chemist	LVR	NEI
Other:	Ling Lee	M.S.	Chemist	LVR	NEI
	L. Carter-Dawson	Ph.D.	Staff Fellow	LVR	NEI
	Gerald J. Chader	Ph.D.	Research Chemist	LVR	NEI

COOPERATING UNITS (if any)

- 1) Bethesda Eye Institute, St. Louis, MO
- 2) Medical College of Wisconsin, Milwaukee, WI

LAB/BRANCH

Laboratory of Vision Research

SECTION

Section on Retinal and Corneal Metabolism

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

2.4

PROFESSIONAL:

1.4

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS

(b) HUMAN TISSUES

(c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

1. A new protein which we call Interphotoreceptor Retinol-Binding Protein (IRBP) has been identified in the subretinal space. Significant light/dark differences in retinol binding to the 7S IRBP and to Cellular Retinol Binding Protein (CRBP) were observed in sub retinal compartments of the rabbit. 7S IRBP isolated from bovine retina has a molecular weight of approximately 243,000. SDS-polyacrylamide gel electrophoresis of this protein revealed a 146,000 MW and a 93,000 MW subunit. Retinol binding to 7S IRBP was first detected at the time of eye opening (13th postnatal day) in the C57 Bl/6 mouse retina.

2. The IRBP could function as a vehicle for vitamin A transport between retina and pigment epithelium. A 14,800 MW unsaturated fatty acid binding protein was purified from developing chick neural retina.

Project Description:

Objectives: To elucidate the mechanism of action of retinoids in ocular tissues and to study their transport between retinal compartments.

Methods Employed: Sucrose density gradient centrifugation, gel filtration, gas chromatography, thin layer chromatography, native disc gel electrophoresis, SDS-polyacrylamide gel electrophoresis, preparative isoelectric focusing and analytical isoelectric focusing on thin layer polyacrylamide gels were employed in studying cellular binding proteins for retinoids and unsaturated fatty acids in ocular tissues.

Major Findings:

1. Interphotoreceptor Retinol-Binding Proteins: Radiolabeled retinol binding was measured in (1) washes of the inner photoreceptor space and surface of the neural retina (SRF/NR) (2) in washes of the apical surface of the retinal pigmented epithelium (SRF/RPE) and (3) the sub-choroidal space in either light- or dark-adapted rabbits. In the SRF/NR there was 3-fold greater retinol binding to the 7S retinol-binding protein (previously detected in outer segments) in the light-adapted state than in the dark-adapted state. There was a similar light/dark difference in 7S retinol binding in SRF/RPE. There was 4.7-fold greater retinol binding to cellular retinol-binding protein (CRBP) in the SRF/RPE, but there was no light/dark difference in the SRF/NR. The specific activity of retinol binding to both the 7S protein and to CRBP was considerably greater in the subretinal compartments than in the supernatant of retinal homogenates. One or both of these proteins may be involved in the transport of vitamin A between NR and RPE.

The 7S retinol-binding protein which we propose to call "Interphotoreceptor Retinol-Binding Protein" has been isolated from bovine retinas by means of gel filtration and native disc gel electrophoresis. Its molecular weight as determined by gradient gel electrophoresis was about 243,000. SDS-polyacrylamide gel electrophoresis of this protein eluted following native disc gel electrophoresis showed the presence of two protein bands, one of 146,000 and another of 93,000 molecular weight.

A developmental study using C57 Bl/6 mice demonstrated that retinol binding to the 7S protein was first detectable in the supernatant fraction of retinal homogenates at the 13th postnatal day, at about the time of eye opening at which time bleaching of rhodopsin occurs.

Fatty Acid Binding: A 14,800 molecular weight unsaturated fatty acid-binding protein was purified to homogeneity from developing chick neural retina and its amino acid composition determined. Extraction of endogenously bound ligands followed by gas chromatographic analysis showed oleic, arachidonic, and palmitoleic acids are bound to this protein.

Significance to Biomedical Research and the Program of the Institute: Vitamin A, in addition to being an essential nutrient for the normal growth and differentiation of most tissues of the body, plays a unique role in the visual process. Retinoid binding proteins are thought to be mediators of vitamin A function. Therefore, studies of the role or roles played by these

proteins, particularly the 7S Interphotoreceptor Retinol-Binding Protein, which may be involved in the transport of retinoids between retinal compartments, will be important in promoting a better understanding of how ocular diseases related to vitamin A metabolism may be prevented or treated.

Proposed Course: Studies will be continued on the role or roles of cellular retinol-binding proteins in mediating the function of vitamin A in ocular tissues.

NEI Research Program: Retinal and Choroidal Diseases--Developmental and Hereditary Disorders

Publications:

Wiggert B, Van Horn DL, Fish BL: Effects of vitamin A deficiency on retinoid binding to cellular retinoid-binding proteins in rabbit cornea and conjunctiva. Exp Eye Res 34:695-702, 1982.

Lai YL, Wiggert B, Liu YP, Chader GJ: Effects of Dark Adaptation on retinol binding in retinal compartments. Invest Ophthalmol Vis Sci 22(suppl):188, 1982.

Lee L, Wiggert B: Characterization of the 7S retinol-binding protein of bovine retina. Invest Ophthalmol Vis Sci 22(suppl):186, 1982.

Carter-Dawson L, Wiggert B, Robison WG: Potential role of the 7S retinol-binding protein in vision. Invest Ophthalmol Vis Sci 22(suppl):249, 1982.

Chader GJ: Retinoids in ocular tissues: binding proteins, transport and mechanism of action, in McDevitt D (ed): Cell Biology of the Eye. New York, Academic Press (in press).

Chader GJ: Vitamin A, in Sears M (ed): Handbook of Experimental Pharmacology. Berlin, Springer-Verlag (in press).

Lai YL, Wiggert B, Liu YP, Chader GJ: Interphotoreceptor retinol-binding proteins: Possible transport vehicles between compartments of the retina. Nature (in press).



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00128-01 LMDB
PERIOD COVERED October 1, 1981, to September 30, 1982			
TITLE OF PROJECT (80 characters or less) Isolation of Type IV Collagen Specific cDNA Clones to Study Eye Morphogenesis			
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT			
PI:	Gabriel Vogeli	Ph.D.	Visiting Scientist
Other:	David Yang	Ph.D.	Guest Worker
	Adriel Bettleheim		Summer Student
			LMDB NEI
			LMDB NEI
			LMDB NEI
COOPERATING UNITS (if any) Mark Sobel, Ph.D., and Marion Young, Ph.D., Laboratory of Developmental Biology and Anomalies, National Institute of Dental Research			
LAB/BRANCH Laboratory of Molecular and Developmental Biology			
SECTION			
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205			
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:	
1.3	1.1	0.2	
CHECK APPROPRIATE BOX(ES)			
<input type="checkbox"/> (a) HUMAN SUBJECTS		<input type="checkbox"/> (b) HUMAN TISSUES	
<input checked="" type="checkbox"/> (c) NEITHER			
<input type="checkbox"/> (a1) MINORS		<input type="checkbox"/> (a2) INTERVIEWS	
SUMMARY OF WORK (200 words or less - underline keywords)			
<p>To study the molecular biology of <u>eye development</u>. I am isolating cDNA clones for high molecular weight lens proteins. <u>Type IV collagen</u> is a major constituent of the lens capsule. Its subunits are coded for by a large messenger RNA of at least 5000 nucleotides. I have constructed a cDNA library in the plasmid pBR322 using high molecular weight RNA (greater than 4500 nucleotides in length) from total chick embryos. This library is screened with <i>in vitro</i> labelled high molecular weight RNA from embryonal chick lenses. Thirty-five cDNA clones from a total of 4200 clones hybridized specifically with the labelled lens RNA. By counter selection with RNA's from different tissues that contain different types of collagen (RNA from cartilage and RNA from chorion-allantois membranes), two prospective type IV collagen specific cDNA clones were selected. These clones are being further analyzed.</p>			

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00127-06 LMDB
PERIOD COVERED October 1, 1981, to September 30, 1982			
TITLE OF PROJECT (80 characters or less) Plasma Membrane Composition and Biosynthesis in Chick Lens Fibers and Epithelia			
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT			
PI:	Peggy Zelenka	Ph.D.	Geneticist LMDB NEI
Other:	Ngoc-Diep Vu	Ph.D.	Staff Fellow LMDB NEI
COOPERATING UNITS (if any) David Beebe, Ph.D., Ass't Professor, USUHS			
LAB/BRANCH Laboratory of Molecular and Developmental Biology			
SECTION			
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205			
TOTAL MANYEARS: 2.05	PROFESSIONAL: 2.05	OTHER: 0.0	
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS			
SUMMARY OF WORK (200 words or less - underline keywords) <p>This project seeks to determine whether the regulation of <u>lens fiber differentiation</u> and maturation is associated with alterations in the plasma membrane. The principal <u>lipid</u> and <u>protein</u> components of embryonic and adult chicken lenses have been identified and their metabolism is being investigated. The relationships between phospholipid metabolism and differentiation have been studied <i>in vivo</i> and <i>in vitro</i> using <u>isotopic labeling techniques</u> and <u>computer modeling</u>. A transient increase in the <u>transmethylation of phosphatidyl-ethanolamine</u> has been shown to be an initial event in lens fiber cell formation. In addition, rapid <u>turnover of phosphatidylinositol</u>, which occurs in lens epithelial cells, ceases abruptly following the initiation of lens fiber formation both <i>in vivo</i> and <i>in vitro</i>.</p>			

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00132-01 LMDB	
PERIOD COVERED October 1, 1981, to September 30, 1982				
TITLE OF PROJECT (80 characters or less) Molecular Biology of Photopigments				
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT				
PI:	Toshimichi Shinohara	Ph.D.	Biologist	LMDB NEI
Others:	Graeme J. Wistow	Ph.D.	Fogarty Fellow	LMDB NEI
	James P. Alligood	B.S.	Biologist	LVR NEI
COOPERATING UNITS (if any)				
LAB/BRANCH Laboratory of Molecular and Developmental Biology				
SECTION				
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205				
TOTAL MANYEARS: 1.25	PROFESSIONAL: 0.75	OTHER: 0.5		
CHECK APPROPRIATE BOX(ES)				
<input type="checkbox"/> (a) HUMAN SUBJECTS		<input type="checkbox"/> (b) HUMAN TISSUES		
<input checked="" type="checkbox"/> (c) NEITHER				
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS				
SUMMARY OF WORK (200 words or less - underline keywords)				
<p>The elucidation of the structure, organization and function of <u>photopigments</u> (<u>rhodopsin</u> and <u>color pigments</u>) and their genes is of fundamental importance. Molecular cloning is a powerful method for studying the function and dysfunction of these visual pigments. Retinal mRNAs from rat, bovine and human eyes were extracted and purified. cDNAs made from the purified mRNAs were cloned by the G-C tailing procedure in the bacterial plasmid pBR322. Rat and bovine recombinant cDNAs libraries were established and are being screened for opsin sequences by hybrid selection and cell-free translation. The nature of the retinal binding site is also under investigation. Retinal binding through an apparent Schiff base linkage to calf γ-crystallin was found.</p>				

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00126-01 LMDB

PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Crystallin Genes: Structure, Organization, Expression, and Evolution

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Joram Piatigorsky	Ph.D.	Chief	LMDB NEI
Others:	J. Fielding Hejtmancik	M.D.	Medical Staff Fellow	LMDB NEI
	Jacques Treton	Ph.D.	Fogarty Fellow	LMDB NEI
	George Inana	M.D.	Research Associate	LMDB NEI
	John M. Nickerson	Ph.D.	Staff Fellow	LMDB NEI
	Raymond E. Jones	Ph.D.	Staff Fellow	LMDB NEI
	Charles R. King	B.S.	Chemist	LMDB NEI
	Leah A. Williams	Ph.D.	Guest Worker	LMDB NEI
	Barbara Norman	B.S.	Chemist	LMDB NEI
	Toshimichi Shinohara	Ph.D.	Biologist	LMDB NEI
	Jin H. Kinoshita	Ph.D.	Scientific Director	NEI
	Deborah A. Carper	B.A.	Biologist	LVR NEI

COOPERATING UNITS (if any)

Joseph Horwitz, Jules Stein Eye Institute, UCLA Medical School; Jacob V. Maizel, Jr., LMG, NICHD; Tom Blundel, Birkbeck College, University of London.

LAB/BRANCH

Laboratory of Molecular and Developmental Biology

SECTION

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
8.0	6.1	1.9

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The structure, organization, expression, and evolution of the genes for the crystallins of the eye lens have been examined. Sequencing of cDNAs for mouse α A- and β -crystallin and chicken δ -crystallin polypeptides have provided primary structures for these lens proteins. The exon-intron structure of a mouse β -crystallin gene was shown to relate to the folding units predicted for its protein and differed greatly from that of the highly interrupted δ -crystallin genes in chickens and ducks. Analysis of cDNAs encoding four mouse γ -crystallin and four chicken β -crystallin polypeptides revealed that these proteins have related gene families which probably arose from a common ancestral β / γ -gene that internally duplicated. A specific deficiency of a 27K β -crystallin mRNA was found in the Philly mouse cataract, and a selective loss of δ -crystallin mRNA was shown in chicken lenses three to five months after hatching. Evidence has been obtained for a new crystallin in the turtle lens. Together, the data extend our knowledge of the molecular genetics of the crystallins.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00146-01 LVR																								
PERIOD COVERED April 5, 1981, to September 30, 1982																											
TITLE OF PROJECT (80 characters or less) Cellular Proliferation in Diabetic Retinopathy																											
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																											
<table> <tr> <td>PI:</td> <td>Leonard M. Hjelmeland</td> <td>Ph.D.</td> <td>Expert Consultant</td> <td>LVR</td> <td>NEI</td> </tr> <tr> <td>Other:</td> <td>J. Bressler</td> <td>Ph.D.</td> <td>Senior Staff Fellow</td> <td>SN</td> <td>NINCDS</td> </tr> <tr> <td></td> <td>G. Grotendorst</td> <td>Ph.D.</td> <td>Staff Fellow</td> <td>LDBA</td> <td>NIDR</td> </tr> <tr> <td></td> <td>A. Chrambach</td> <td>Ph.D.</td> <td>Research Chemist</td> <td>RR</td> <td>NICHD</td> </tr> </table>				PI:	Leonard M. Hjelmeland	Ph.D.	Expert Consultant	LVR	NEI	Other:	J. Bressler	Ph.D.	Senior Staff Fellow	SN	NINCDS		G. Grotendorst	Ph.D.	Staff Fellow	LDBA	NIDR		A. Chrambach	Ph.D.	Research Chemist	RR	NICHD
PI:	Leonard M. Hjelmeland	Ph.D.	Expert Consultant	LVR	NEI																						
Other:	J. Bressler	Ph.D.	Senior Staff Fellow	SN	NINCDS																						
	G. Grotendorst	Ph.D.	Staff Fellow	LDBA	NIDR																						
	A. Chrambach	Ph.D.	Research Chemist	RR	NICHD																						
COOPERATING UNITS (if any)																											
1) Reproductive Research Branch, NICHD 2) Surgical Neurology Branch, NINCDS 3) Laboratory of Developmental Biology and Anomalies, NIDR 4) Wilmer-Woods Institute, Johns Hopkins University Hospital																											
LAB/BRANCH																											
Laboratory of Vision Research																											
SECTION																											
Section on Retinal and Corneal Metabolism																											
INSTITUTE AND LOCATION																											
National Eye Institute, NIH, Bethesda, Maryland 20205																											
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:																									
0.3	0.3	0.0																									
CHECK APPROPRIATE BOX(ES)																											
<input type="checkbox"/> (a) HUMAN SUBJECTS		<input checked="" type="checkbox"/> (b) HUMAN TISSUES																									
<input type="checkbox"/> (a1) MINORS		<input type="checkbox"/> (a2) INTERVIEWS																									
□ (c) NEITHER																											
SUMMARY OF WORK (200 words or less - underline keywords)																											
<p>Research is being conducted on the biological mechanisms which control proliferation of various cell types in <u>diabetic retinopathy</u> which include <u>fibroblasts</u>, <u>glia</u>, <u>pigment epithelium</u>, and <u>vascular smooth muscle</u>. Using <u>wound repair</u> as a general model, we have demonstrated that glial cells exhibit <u>chemotaxis to platelet-derived growth factor (PDGF)</u> in a fashion similar to that described in previous reports on smooth muscle and fibroblasts. Pigmented epithelial cells are currently being evaluated for chemotaxis to PDGF. As a consequence of the general wound repair model, each of these cell types is, in turn, being evaluated as a possible source of soluble factors which may be involved in <u>neovascularization</u>.</p>																											

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00145-01 LVR
PERIOD COVERED October 1, 1981, to September 30, 1982			
TITLE OF PROJECT (80 characters or less) Effects of Aging and Nutrition on the Retina and Retinal Pigment Epithelium			
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT			
PI: Martin L. Katz Ph.D. Staff Fellow LVR NEI Other: W. Gerald Robison, Jr. Ph.D. Chief, Section on Experimental Anatomy LVR NEI			
COOPERATING UNITS (if any)			
LAB/BRANCH Laboratory of Vision Research			
SECTION Section on Experimental Anatomy			
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205			
TOTAL MANYEARS: 0.6	PROFESSIONAL: 0.4	OTHER: .2	
CHECK APPROPRIATE BOX(ES)			
<input type="checkbox"/> (a) HUMAN SUBJECTS		<input type="checkbox"/> (b) HUMAN TISSUES	
<input type="checkbox"/> (a1) MINORS		<input type="checkbox"/> (a2) INTERVIEWS	
<input checked="" type="checkbox"/> (c) NEITHER			
<p>SUMMARY OF WORK (200 words or less - underline keywords)</p> <p>Investigations were carried out to characterize <u>senescent changes</u> in the pigmented rat <u>retina</u> and <u>retinal pigment epithelium</u> (RPE) using <u>morphological</u> and <u>biochemical</u> techniques. Age related losses of cells from the <u>photoreceptor</u>, <u>inner nuclear</u>, and <u>ganglion cell layers</u> were found. <u>Rhodopsin</u> levels were found to change very little during aging. Senescent changes in the RPE included: (1) a progressive accumulation of <u>lipofuscin</u>; (2) a shortening and thickening of the <u>apical microvilli</u>; (3) modification and enlargement of the <u>basal infoldings</u>; and (4) accumulation of electron-dense material between the basal infoldings. We have found that the drug <u>centrophenoxine</u> does not reverse age-related lipofuscin accumulation in the RPE. <u>Acid phosphatase</u> levels in the RPE did not change during senescence. We are currently performing measurements to determine whether aging has an affect on <u>photoreceptor disc shedding</u> and <u>phagocytosis</u> by the RPE. We will also determine whether <u>vitamin A</u> uptake and esterification by the RPE during light adaptation are altered during senescence. Finally, we are characterizing the <u>apical membrane proteins</u> of the RPE, and will determine whether these proteins are altered with age.</p>			

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00129-10 LVR
PERIOD COVERED October 1, 1981, to September 30, 1982			
TITLE OF PROJECT (80 characters or less) Anatomical and Pathological Studies of Ocular Tissues			
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT			
PI:	Toichiro Kuwabara	M.D.	Chief, Section on Experimental Pathology LVR NEI
Other:	Yasumichi Yajima	M.D.	Visiting Scientist LVR NEI
	Yoshio Akagi	M.D.	Visiting Scientist LVR NEI
	Douglas Sisk	M.S.	Predoctoral Fellow LVR NEI
COOPERATING UNITS (if any)			
LAB/BRANCH Laboratory of Vision Research			
SECTION Section on Experimental Pathology			
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205			
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:	
3.1	2.1	1.0	
CHECK APPROPRIATE BOX(ES)			
<input type="checkbox"/> (a) HUMAN SUBJECTS		<input checked="" type="checkbox"/> (b) HUMAN TISSUES	
		<input type="checkbox"/> (c) NEITHER	
<input type="checkbox"/> (a1) MINDRS <input type="checkbox"/> (a2) INTERVIEWS			
SUMMARY OF WORK (200 words or less - underline keywords)			
<p><u>Histopathological studies</u> were conducted on numerous human and animal eyes by transmission and scanning <u>electron microscopy</u>, as well as by <u>histochemistry</u> and <u>histology</u>. In studies designed to determine the etiology of <u>gyrate atrophy</u>, intravitreal injection of <u>L-ornithine hydrochloride</u> in physiologic saline solution caused marked edema specifically in the <u>pigment epithelium</u> of Sprague-Dawley strain albino and Evans black hooded rats and rhesus and cynomologus monkeys. Swelling of the pigment epithelial cells, which was most prominent four hours after the injection, disappeared by 24 hours. However, many pigment epithelial cells gradually degenerated resulting in patches of denuded areas. The <u>photoreceptor cells</u> overlying the damaged pigment epithelium degenerated secondarily.</p>			

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00012-03 LVR
PERIOD COVERED October 1, 1981 to September 30, 1982			
TITLE OF PROJECT (80 characters or less) Growth of the Retinal Pigment Eptiehlium			
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT			
PI: Alfred J. Coulombre		Ph.D.	Chief, Section on Experimental Embryology LVR NEI
Other: None			
COOPERATING UNITS (if any)			
LAB/BRANCH Laboratory of Vision Research			
SECTION Section on Experimental Embryology			
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205			
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:	
1.0	1.0	0.0	
CHECK APPROPRIATE BOX(ES)			
<input type="checkbox"/> (a) HUMAN SUBJECTS		<input type="checkbox"/> (b) HUMAN TISSUES	
		<input checked="" type="checkbox"/> (c) NEITHER	
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS			
SUMMARY OF WORK (200 words or less - underline keywords)			
<p>The formation of <u>neural retina</u> (NR) from retinal <u>pigmented epithelium</u> (RPE) of chick embryos in culture was investigated. In cultures of explants of RPE, depigmented, <u>preretinal foci</u>, consisting of 50 to 100 cells appeared in the pigmented central portion of the explant within three days. Then these <u>depigmented cells</u> increased rapidly in number and by about day 14 they formed characteristic spherical bodies, which were identified as a neural retinal-like structure (NR structure) by electron microscopic observations. Culture of explants of RPE from embryos of different stages showed that the capacity of embryonic RPE to form an NR structure decreased steadily with embryonic age from stage 24 to 27. At and after stage 27, no foci leading to the neural retinal <u>differentiation</u> were formed in the explants. Medium conditioned by cell cultures of chicken embryonic NR, RPE, or chondrocytes had no effect on the formation of NR structures by explants of RPE.</p>			

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00070-05 LVR		
PERIOD COVERED October 1, 1981, to September 30, 1982					
TITLE OF PROJECT (80 characters or less) Vitamin A and Ocular Tissues					
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT					
PI:	Barbara Wiggert	Ph.D.	Research Chemist	LVR	NEI
Other:	Ling Lee	M.S.	Chemist	LVR	NEI
	L. Carter-Dawson	Ph.D.	Staff Fellow	LVR	NEI
	Gerald J. Chader	Ph.D.	Research Chemist	LVR	NEI
COOPERATING UNITS (if any)					
1) Bethesda Eye Institute, St. Louis, MO 2) Medical College of Wisconsin, Milwaukee, WI					
LAB/BRANCH Laboratory of Vision Research					
SECTION Section on Retinal and Corneal Metabolism					
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205					
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:			
2.4	1.4	1.0			
CHECK APPROPRIATE BOX(ES)					
<input type="checkbox"/> (a) HUMAN SUBJECTS		<input type="checkbox"/> (b) HUMAN TISSUES			
		<input checked="" type="checkbox"/> (c) NEITHER			
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS					
SUMMARY OF WORK (200 words or less - underline keywords)					
<p>1. A new protein which we call <u>Interphotoreceptor Retinol-Binding Protein</u> (IRBP) has been identified in the <u>subretinal space</u>. Significant <u>light/dark differences in retinol binding</u> to the <u>7S IRBP</u> and to <u>Cellular Retinol Binding Protein</u> (CRBP) were observed in sub retinal compartments of the rabbit. <u>7S IRBP</u> isolated from <u>bovine retina</u> has a <u>molecular weight</u> of approximately <u>243,000</u>. SDS-polyacrylamide gel electrophoresis of this protein revealed a <u>146,000 MW</u> and a <u>93,000 MW</u> subunit. <u>Retinol binding to 7S IRBP</u> was first detected at the time of eye opening (<u>13th postnatal day</u>) in the <u>C57 Bl/6 mouse retina</u>.</p> <p>2. The IRBP could function as a vehicle for vitamin A transport between retina and pigment epithelium. A <u>14,800 MW unsaturated fatty acid binding protein</u> was <u>purified from developing chick neural retina</u>.</p>					

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00015-17 LVR

PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

The Cell Biology of the Vertebrate Retina

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Paul J. O'Brien	Ph.D.	Research Chemist	LVR	NEI
Other:	James P. Alligood	B.S.	Biologist	LVR	NEI
	Nancy Philp	Ph.D.	Post-Doctoral Fellow	LVR	NEI
	Peter A. Dudley	Ph.D.	Staff-Fellow	LVR	NEI

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Vision Research

SECTION

Section on Cell Biology

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

1.6

PROFESSIONAL:

1.4

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Opsin labeling with galactose was found to occur at a constant rate throughout the diurnal cycle. Thus, neither synthesis nor intracellular transport of opsin appears to exhibit any circadian rhythm.

Iodination of bovine pigment epithelium followed by SDS gel electrophoresis revealed a reproducible pattern of plasma membrane proteins, several of which were also labeled on incubation of eyecups with radioactive glucosamine. Thus these are glycoproteins which may mediate recognition of outer segment membranes in preparation for phagocytosis.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00016-15 LVR
PERIOD COVERED October 1, 1981, to September 30, 1982			
TITLE OF PROJECT (80 characters or less) The Biochemistry of Normal and Dystrophic Retinas			
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT			
PI:	Paul J. O'Brien	Ph.D.	Research Chemist LVR NEI
Other:	James P. Alligood	B.S.	Biologist LVR NEI
	Nancy Philp	Ph.D.	Post-Doctoral Fellow LVR NEI
COOPERATING UNITS (if any) School of Veterinary Medicine, University of Pennsylvania			
LAB/BRANCH Laboratory of Vision Research			
SECTION Section on Cell Biology			
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205			
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:	
0.9	0.7	0.2	
CHECK APPROPRIATE BOX(ES)			
<input type="checkbox"/> (a) HUMAN SUBJECTS		<input type="checkbox"/> (b) HUMAN TISSUES	
<input checked="" type="checkbox"/> (c) NEITHER			
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS			
SUMMARY OF WORK (200 words or less - underline keywords) <u>Opsin synthesis</u> was measured in <u>miniature poodles</u> affected with <u>progressive rod-cone degeneration</u> . Photoreceptors develop normally but begin to degenerate after the dog is fully grown. At all ages studied the rate of <u>rod outer segment renewal</u> was about half the normal value. <u>Opsin synthesis</u> , however, occurred at the normal rate until advanced stages of the disease when photoreceptor cell death was apparent. Thus the defect may involve <u>photoreceptor membrane assembly</u> rather than synthesis.			



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00148-09 LVR	
PERIOD COVERED October 1, 1981, to September 30, 1982				
TITLE OF PROJECT (80 characters or less) Cyclic Nucleotides and Vision				
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT				
PI:	Gerald J. Chader Susan Gentleman	Ph.D. Ph.D.	Research Chemist Expert	LVR NEI LVR NEI
Other:	R. T. Fletcher Robert L. Somers	M.S. B.S.	Chemist Chemist	LVR NEI LVR NEI
COOPERATING UNITS (if any) 1) Section on Ophthalmology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 2) Department of Anatomy, Erasmus University, Rotterdam, The Netherlands				
LAB/BRANCH Laboratory of Vision Research				
SECTION Section on Retinal and Corneal Metabolism				
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205				
TOTAL MANYEARS:	3.5	PROFESSIONAL: 1.5	OTHER: 2.0	
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS				
SUMMARY OF WORK (200 words or less - underline keywords) <p>1) Distinct biochemical differences have been found between models for <u>early onset retinal degeneration</u> that were previously thought to belong to a single disease type. Although <u>cyclic nucleotide</u> metabolism is deranged in each animal model, quantitative and qualitative differences are observed in enzymes involved in regulating the concentration of both <u>cAMP</u> and <u>cGMP</u>. This indicates that there may be multiple etiologies of these diseases and that the causes of each disease must be separately established for each animal species or genetic variation.</p> <p>2) The role of cyclic nucleotides in visual cells other than the photoreceptor visual process appears to be complex having some similarities to well known brain fractions but also some distinct and potentially unique differences. cAMP in particular appears to be involved in <u>retinomotor movements</u> in <u>retina</u> and <u>pigment epithelium</u>.</p>				

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00124-02 LVR

PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Metabolism of the Retina and Pigment Epithelium

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Shay-Whey M. Koh	Ph.D.	Staff Fellow	LVR	NEI
	Gerald J. Chader	Ph.D.	Research Chemist	LVR	NEI
Other:	A. Kyritsis	M.D.	Visiting Scientist	LVR	NEI

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Vision Research

SECTION

Section on Retinal and Corneal Metabolism

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

2.75

PROFESSIONAL:

2.75

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Characteristics of metabolism and function of the neural retina (NR) and pigment epithelium (PE) are studied in cultured cells and in freshly dissected tissue. Several new neurotransmitter/neuromodulator receptors have been identified in retina and pigment epithelial cells in culture. For example, PE cells respond to VIP, prostaglandins, glucagon and β -adrenergic agonists. Neural retinal cells respond to glucagon, VIP and dopamine. These substances may control important processes of NR and PE cells *in vivo*.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00066-05 LVR
PERIOD COVERED October 1, 1981, to September 30, 1982			
TITLE OF PROJECT (80 characters or less) Neurotransmitter Chemistry of Retinal Neurons			
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT			
PI: Barbara-Anne Battelle	Ph.D.	Senior Staff Fellow	LVR NEI
Other: Judith A. Evans	Ph.D.	Staff Fellow	LVR NEI
Donald G. Puro	M.D.	Medical Officer	LVR NEI
COOPERATING UNITS (if any) State University of New York, Stoney Brook, NY Institute for Sensory Research, Syracuse University, Syracuse, NY Marine Biological Laboratory, Woods, Hole, MA			
LAB/BRANCH Laboratory of Vision Research			
SECTION Section on Cell Biology			
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205			
TOTAL MANYEARS: 2	PROFESSIONAL: 2	OTHER: 0.0	
CHECK APPROPRIATE BOX(ES)			
<input type="checkbox"/> (a) HUMAN SUBJECTS		<input type="checkbox"/> (b) HUMAN TISSUES	
		<input checked="" type="checkbox"/> (c) NEITHER	
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS			
SUMMARY OF WORK (200 words or less - underline keywords)			
<p>A combined <u>biochemical</u> and <u>anatomical</u> study is underway to identify <u>chemical neurotransmitters</u> in retinal neurons and in neurons of the visual pathway, to examine the <u>development of neurotransmitter systems</u> in retina, and to determine the <u>role of chemical neurotransmitters</u> in the processing of visual information. Two systems are being investigated: (1) the relatively simple visual system of <u>Limulus polyphemus</u> and (2) developing <u>mammalian retinal neurons</u> in intact retinas and in <u>monolayer cell culture</u>. Work with the simple visual system has led to the identification of the biogenic amine <u>octopamine</u> as a neurotransmitter released from <u>retinal efferent fibers</u>; i.e. fibers which originate from cells in the brain and innervate the retina. Our studies suggest that this amine may alter <u>visual sensitivity</u> and may be important in maintaining normal photoreceptor cell function. Studies of the intact developing retina have shown that at least two neurotransmitters, <u>GABA</u> and <u>acetylcholine</u> appear very early in normal retinal development. The regulation of development of these two neurotransmitter systems is being investigated using monolayer cell cultures.</p>			



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00149-09 LVR		
PERIOD COVERED October 1, 1981, to September 30, 1982					
TITLE OF PROJECT (80 characters or less) Ultrastructure and Function of the Pigment Cells of the Eye					
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT					
PI:	W. Gerald Robison, Jr.	Ph.D.	Chief, Section on Experimental Anatomy	LVR	NEI
Other:	Toichiro Kuwabara	M.D.	Chief, Section on Experimental Pathology	LVR	NEI
	Martin L. Katz	Ph.D.	Staff Fellow	LVR	NEI
	Roland K. Herrmann	M.D.	Visiting Fellow	LVR	NEI
	John G. Bieri	Ph.D.	Chief, Section on Nutritional Biochemistry	LNE	NIADDK
COOPERATING UNITS (if any) Laboratory of Nutrition and Endocrinology, NIADDK					
LAB/BRANCH Laboratory of Vision Research					
SECTION Section on Experimental Anatomy					
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205					
TOTAL MANYEARS:	2.1	PROFESSIONAL:	1.2	OTHER:	0.9
CHECK APPROPRIATE BOX(ES)					
<input type="checkbox"/> (a) HUMAN SUBJECTS		<input type="checkbox"/> (b) HUMAN TISSUES		<input checked="" type="checkbox"/> (c) NEITHER	
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS					
SUMMARY OF WORK (200 words or less - underline keywords) Intracellular accumulations of <u>lipofuscin</u> (<u>aging pigment</u>) occur gradually with age and can be accelerated by <u>vitamin E deficiency</u> as a model for this aspect of aging. Supposedly, lipofuscin originates from highly oxidized cell products which polymerize and form insoluble, <u>autofluorescent granules</u> within the cytoplasm. Our studies confirm the classical concept that membrane lipids containing <u>polyunsaturated fatty acids</u> (PUFA) contribute significantly to lipofuscin formation in the <u>retinal pigment epithelium</u> (RPE). However, dietary levels of <u>vitamin A</u> appear to be even more important in determining the rate of lipofuscin accumulation. Even a <u>RPE</u> with no source of ingestable <u>rod outer segment membranes</u> which contain PUFA will accumulate large amounts of lipofuscin in a relatively short time if the level of vitamin A is high, but will accumulate almost none if vitamin A is absent. In fact, the RPE of rats fed different amounts of vitamin A (23, 2.3, .23, .058 and 0.0 mg retinol/kg diet) showed a dose-related response with respect to the amount of lipofuscin accumulated. Thus, for the first time, it appears that vitamin A has a direct involvement in lipofuscin formation, and even may become included in lipofuscin granules as one of the oxidized byproducts of cell metabolism.					



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00032-06 LVR		
PERIOD COVERED October 1, 1981, to May 12, 1982					
TITLE OF PROJECT (80 characters or less) Role of Vitamin A in Maintenance and Development of Ocular Tissues					
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT					
PI:	Louvenia Carter-Dawson	Ph.D.	Staff Fellow	LVR	NEI
Other:	W. Gerald Robison, Jr.	Ph.D.	Chief, Section on Experimental Anatomy	LVR	NEI
	Toichiro Kuwabara	M.D.	Chief, Section on Experimental Pathology	LVR	NEI
	Barbara Wiggert	Ph.D.	Research Chemist	LVR	NEI
	John G. Bieri	Ph.D.	Chief, Section on Nutritional Biochemistry	LNE	NIADDK
COOPERATING UNITS (if any) Laboratory of Nutrition and Endocrinology, NIADDK					
LAB/BRANCH Laboratory of Vision Research					
SECTION Section on Experimental Anatomy					
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205					
TOTAL MANYEARS:	1.3	PROFESSIONAL:	1.2	OTHER:	0.1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS					
SUMMARY OF WORK (200 words or less - underline keywords) <p>The neural retina has a soluble <u>7S retinol-binding protein</u> which was described previously (Wiggert et al., 1976). To elucidate more precisely the role of this protein in <u>vision</u> we examined developmental changes in the amounts of tritiated <u>retinol</u> bound to the <u>7S protein</u> and compared these to <u>rhodopsin levels</u> and <u>retinal structure</u> in the <u>C57BL/6 mouse</u>. Binding of tritiated retinol by the <u>7S protein</u> is not detected in retinal cytosol until the 13th postnatal day (P13). Approximately 0.4 pmoles of retinol/mg cytosol protein is bound at P13. A 6-10 fold increase in amount of retinol bound is seen over the next seven days reaching adult levels of 9-12 pmoles of retinol bound/mg cytosol protein around P40. The initial detection of tritiated retinol binding to the <u>7S protein</u> does not coincide with the appearance of <u>outer segments</u> or <u>rhodopsin</u> which are detectable at P5 and P7 respectively. However, detection of tritiated retinol binding to this protein does coincide with eye opening at which time bleaching of rhodopsin occurs. The detection of the <u>7S retinol-binding protein</u> at this stage of visual maturation suggests it may play a role in the cycle of rhodopsin bleaching and regeneration.</p>					

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00023-04 LVR

PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Macrophage Interactions with Other Cells and Their Products

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Igal Gery	Ph.D.	Head, Section on Experimental Immunology	LVR	NEI
Other:	Jose-Luis Lepe-Zuniga	M.D.	Visiting Fellow	LVR	NEI
	Manabu Mochizuki	M.D.	Visiting Associate	LVR	NEI
	J. Samuel Zigler, Jr.	Ph.D.	Research Biologist	LVR	NEI
	Robert B. Nussenblatt	M.D.	Head, Section on Ophthalmic Immunology	CB	NEI
	John A. Schmidt	M.D.	Research Associate	LI	NIAID
	Julia Derr	B.S.	Biologist	LVR	NEI

COOPERATING UNITS (if any)

Laboratory of Immunology, NIAID

LAB/BRANCH

Laboratory of Vision Research

SECTION

Section on Experimental Immunology

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.6	1.8	0.8

CHECK APPROPRIATE BOX(ES)

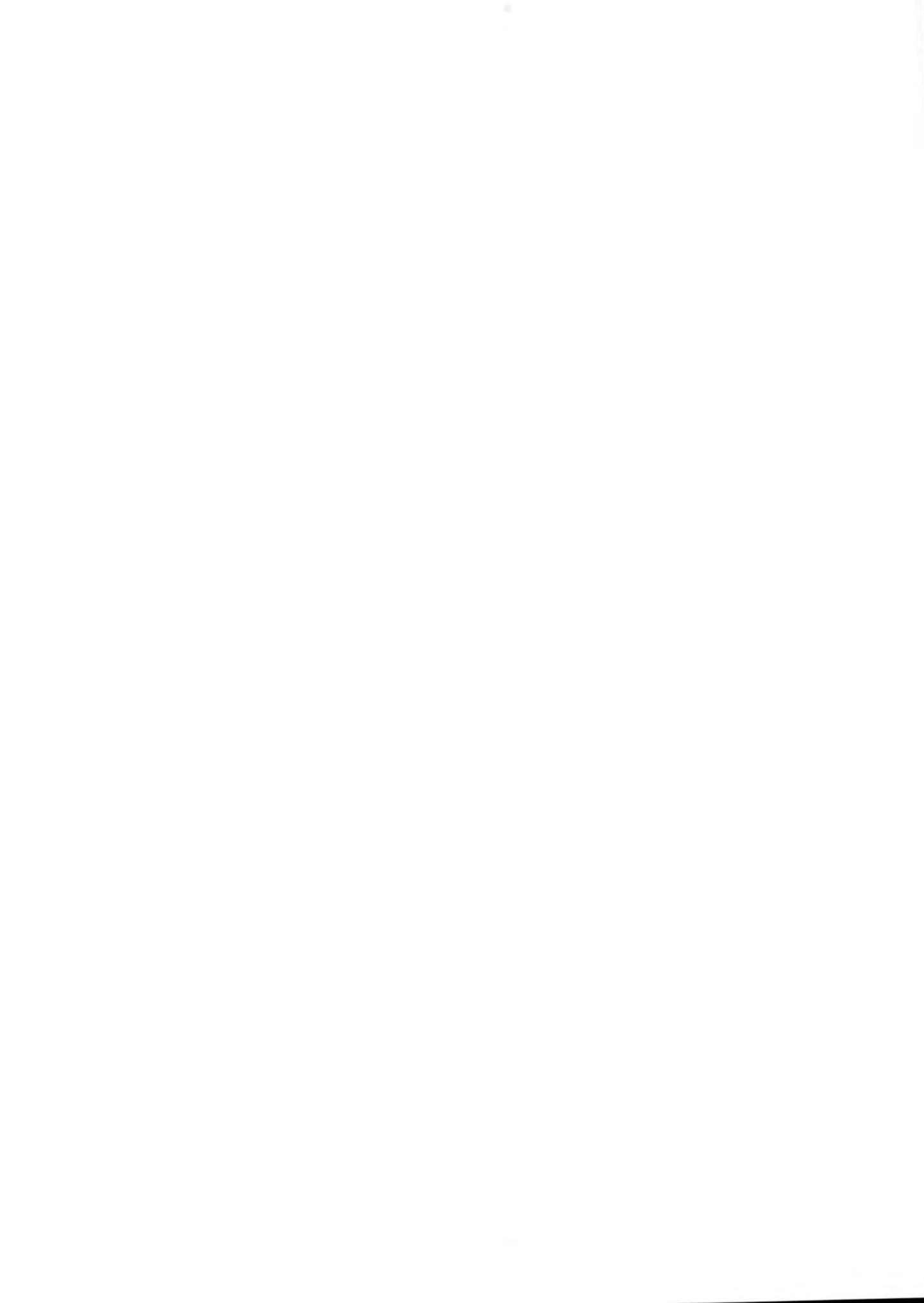
(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Various aspects of macrophage interactions with ocular and other cells were examined. (1) Activated macrophages, which are a major component of inflammatory reactions, may affect the metabolism of surrounding tissues. This damaging effect was further studied by using lens epithelial cells as the target. Of importance is the finding that prostaglandins regulate this macrophage activity, without acting directly as the damaging mediators. (2) Two macrophage-made mediators which have similar properties are interleukin 1, which activates lymphocytes, and a factor that stimulates fibroblasts. The possibility that these two factors are identical was previously proposed and supported by some preliminary data. This notion has been further supported by a series of experiments showing that the two mediators have multiple identical physical and biochemical properties. (3) Interleukin 1, which mediates certain immune responses, has been used to examine the mode of action of a newly introduced immunosuppressive drug, cyclosporin A. The drug was found to inhibit strongly certain responses mediated by interleukin 1, but to have minimal effect on other responses stimulated by this mediator.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00069-05 LVR
PERIOD COVERED <u>October 1, 1981, to September 30, 1982</u>			
TITLE OF PROJECT (80 characters or less) <u>Immune Responses to Ocular Antigens</u>			
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT			
PI:	Igal Gery	Ph.D. Chief, Section on Experimental Immunology	LVR NEI
Other:	Robert B. Nussenblatt	M.D. Chief, Section on Ophthalmic Immunology	CB NEI
	Manabu Mochizuki	M.D. Visiting Associate	LVR NEI
	W. Gerald Robison, Jr.	Ph.D. Chief, Section on Experimental Anatomy	LVR NEI
	Julia Derr	B.S. Biologist	LVR NEI
COOPERATING UNITS (if any)			
1) Dept. of Medicine, Northwestern University Med. School, Chicago, IL 2) Institute for Biological Sciences, Oakland Univ. Rochester, MI			
LAB/BRANCH			
Laboratory of Vision Research			
SECTION			
Section on Experimental Immunology			
INSTITUTE AND LOCATION			
National Eye Institute, NIH, Bethesda, Maryland 20205			
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:	
1.5	1.3	0.2	
CHECK APPROPRIATE BOX(ES)			
<input type="checkbox"/> (a) HUMAN SUBJECTS		<input type="checkbox"/> (b) HUMAN TISSUES	
		<input checked="" type="checkbox"/> (c) NEITHER	
<input type="checkbox"/> (a1) MINDRS <input type="checkbox"/> (a2) INTERVIEWS			
SUMMARY OF WORK (200 words or less - underline keywords)			
<p>Mechanisms involved in the development of <u>pathogenic autoimmune processes</u> in the eye have been studied by using an animal disease, <u>experimental autoimmune uveitis (EAU)</u>. This disease is considered a possible model for certain <u>human ocular diseases</u>. Findings of specific interest are:</p> <p>(1) transfer of EAU to naive rats was achieved with highest efficacy when <u>lymph node cells</u> from donors with EAU were used, following preincubation with the sensitizing <u>S-antigen</u>. (2) The transferred EAU was <u>prevented</u> by treating the recipient rats with the <u>immunosuppressive drug, cyclosporin A</u>. This finding indicates that <u>recruitment</u> and <u>clone expansion</u> are essential for the development of EAU. (3) Studies with rats of <u>inbred strains</u> with different <u>genetic makeup</u> were extended, and confirmed our preliminary observation that genetic factors affect the susceptibility of rats to EAU. In addition, it was found that the development of disease in relatively resistant strains of rats was significantly enhanced by treatment with <u>Bordetella pertussis bacteria</u>, thus indicating the important role of <u>environmental factors</u> in determining an individual's susceptibility to EAU.</p>			



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00125-02 LVR
PERIOD COVERED October 1, 1981, to September 30, 1982			
TITLE OF PROJECT (80 characters or less) Neuropharmacology of the Retina			
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT			
PI:	Donald G. Puro	M.D., Ph.D.	Medical Officer Ophthalmologist LVR NEI
Other:	Hermes H. Yeh Barbara-Anne Battelle	Ph.D. Ph.D.	Staff Fellow Senior Staff Fellow CB NEI LVR NEI
COOPERATING UNITS (if any) None			
LAB/BRANCH Laboratory of Vision Research			
SECTION Section on Experimental Biology			
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, MD 20205			
TOTAL MANYEARS:	2.0	PROFESSIONAL:	2.0 OTHER: 0.0
CHECK APPROPRIATE BOX(ES)			
<input type="checkbox"/> (a) HUMAN SUBJECTS		<input type="checkbox"/> (b) HUMAN TISSUES	
<input type="checkbox"/> (a1) MINORS		<input type="checkbox"/> (a2) INTERVIEWS	
<p>SUMMARY OF WORK (200 words or less - underline keywords)</p> <p>Knowledge basic to the development of a <u>pharmacological approach</u> to the prevention and treatment of <u>retinal disorders</u> is being acquired. A combination of technological advances in <u>neuropharmacology</u>, <u>intracellular electrophysiology</u> and <u>cell biology</u>, including <u>cell culture</u>, is used to explore the actions and interactions of <u>neurotransmitters</u>, <u>neuromodulators</u>, <u>hormones</u> and selected <u>drugs</u> on specific types of retinal neurons.</p>			

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00005-10 LVR

PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Electrophysiology and Morphology of Mammalian and Avian Retinas

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Ralph Nelson	Ph.D.	Physiologist	LVR	NEI
	Avery Nelson	Ph.D.	Senior Staff Fellow	LVR	NEI
	Andrew Mariani	Ph.D.	Staff Fellow	LVR	NEI

COOPERATING UNITS (if any)

Department of Physiology, University of Utah, Salt Lake City
Max-Planck-Institut fur Physiologische und Klinische Forshung, Bad
Nauheim, Federal Republic of Germany

LAB/BRANCH
Laboratory of Vision Research

SECTION
Section on Experimental Biology

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 2.5 PROFESSIONAL: 2.5 OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We study the interactions among and interconnections between retinal neurons using electrophysiological and anatomical techniques. Neurons in cat, monkey, or pigeon retina are stained by the Golgi technique or by direct, intracellular injection of horseradish peroxidase (HRP) and subsequent histochemical reactions. The extents of dendritic and axonal arborizations of morphologically identified cells are illustrated in camera lucida drawings. Electrophysiological response properties are determined by intracellular recording. Electron microscopy reveals the synaptic relationships between photoreceptor, horizontal, bipolar, amacrine, and ganglion cell types. These data allow the inference of neural circuits and functional units within the retina and demonstrate the correlations between neural structure and function.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00105-03 LVR
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PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (50 characters or less)

Structure and Composition of Lens Crystallins with Respect to Cataract Development

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Samuel Zigler, Jr.	Ph.D	Research Biologist	LVR	NEI
Other:	Richard Bodaness	M.D., Ph.D.	Senior Staff Fellow	LVR	NEI
	Tien-sheng Hu	M.D.	Guest Worker	LVR	NEI
	Igal Gery	Ph.D.	Visiting Scientist	LVR	NEI

COOPERATING UNITS (if any)

Howard M. Jernigan, Jr., Univ. of Tennessee Center for Health Sciences

LAB/BRANCH
Laboratory of Vision Research

SECTION
Section on Lens and Cataract

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	1.9	PROFESSIONAL:	1.9	OTHER:	0
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CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It is known that oxidative stress leads to a number of well-characterized structural modifications in lens crystallins. These modified lens proteins accumulate within the lens and may play a major role in formation of some senile cataracts. We have proposed that singlet oxygen produced photodynamically may account for the oxidative damage. We have shown that photosensitizers endogenous to the lens can support the process, as could photosensitizing drugs which reach the lens. We have recently been able to extend studies of possible oxidative mechanisms of cataractogenesis to cataracts associated with chronic uveitis and retinal degenerative diseases respectively. In the first case, the lens is exposed to macrophages which are known to produce active species of oxygen, particularly hydrogen peroxide and superoxide. Cultured lenses are damaged when exposed to activated macrophages, apparently by an oxidative mechanism. In the case of cataracts associated with retinal degeneration we believe that peroxidation of the polyunsaturated lipids from degenerating rod outer segments may generate toxic species which initiate cataract.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00136-10 LVR																									
PERIOD COVERED October 1, 1981, to September 30, 1982																												
TITLE OF PROJECT (80 characters or less) Chemistry and Metabolism of the Lens																												
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																												
<table> <tr> <td>PI:</td> <td>Paul Russell</td> <td>Ph.D.</td> <td>Research Chemist</td> <td>LVR</td> <td>NEI</td> </tr> <tr> <td>Other:</td> <td>Tien-sheng Hu</td> <td>M.D.</td> <td>Visiting Scientist</td> <td>LVR</td> <td>NEI</td> </tr> <tr> <td></td> <td>Lorenzo Merola</td> <td>M.S.</td> <td>Chemist</td> <td>LVR</td> <td>NEI</td> </tr> <tr> <td></td> <td>Geeta Pararajasegaram</td> <td>B.S.</td> <td>Guest Worker</td> <td>LVR</td> <td>NEI</td> </tr> </table>					PI:	Paul Russell	Ph.D.	Research Chemist	LVR	NEI	Other:	Tien-sheng Hu	M.D.	Visiting Scientist	LVR	NEI		Lorenzo Merola	M.S.	Chemist	LVR	NEI		Geeta Pararajasegaram	B.S.	Guest Worker	LVR	NEI
PI:	Paul Russell	Ph.D.	Research Chemist	LVR	NEI																							
Other:	Tien-sheng Hu	M.D.	Visiting Scientist	LVR	NEI																							
	Lorenzo Merola	M.S.	Chemist	LVR	NEI																							
	Geeta Pararajasegaram	B.S.	Guest Worker	LVR	NEI																							
COOPERATING UNITS (if any)																												
LAB/BRANCH Laboratory of Vision Research																												
SECTION Section on Lens and Cataract																												
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205																												
TOTAL MANYEARS:	4.0	PROFESSIONAL:	3.0	OTHER:																								
CHECK APPROPRIATE BOX(ES)																												
<input type="checkbox"/> (a) HUMAN SUBJECTS		<input checked="" type="checkbox"/> (b) HUMAN TISSUES		<input type="checkbox"/> (c) NEITHER																								
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																												
SUMMARY OF WORK (200 words or less - underline keywords)																												
<p>Investigation into the alterations in <u>protein composition</u> of the <u>lens</u> were conducted with particular reference to <u>membrane proteins</u>. Development and refinement of <u>in vitro model systems</u> for <u>cataract development</u> have continued. Results from these <u>in vitro</u> systems indicate many of the steps during cataract development proceed in a very similar manner regardless of the <u>initiating event</u> in cataractogenesis. <u>Glyceraldehyde phosphate dehydrogenase</u>, an enzyme in the <u>glycolytic pathway</u>, has been studied <u>in vivo</u> and <u>in vitro</u>. The possible <u>inactivation</u> of the enzyme during cataract formation has been under investigation. Cultures of <u>lens epithelial cells</u> as well as other cells from <u>tissue culture</u> have been utilized to study <u>basement membrane formation</u>, <u>cytotoxic agents</u>, and enzymes such as <u>aldose reductase</u>. These cells are beneficial because they facilitate the study of biological events in a well-defined and easily manipulated system.</p>																												

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00003-10 LVR

PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Cataracts

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Peter Kador	Ph.D.	Research Chemist	LVR	NEI
Other:	Jin H. Kinoshita	Ph.D.	Scientific Director		NEI
	Deborah A. Carper	B.A.	Biologist	LVR	NEI
	Roland Herrmann	M.D.	Visiting Fellow	LVR	NEI
	Yoshio Akagi	M.D.	Visiting Scientist	LVR	NEI

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Vision Research

SECTION

Section on Lens and Cataract

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.3	2.3	1.0

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Current investigations are being conducted on the events leading to the formation of several types of cataracts. Diabetic or sugar cataract formation initiated by the enzyme aldose reductase is being studied. Methods for controlling the onset of these cataracts through the regulation of this enzyme are being developed. Hereditary cataract formation is also being studied in a strain of mice developed in our laboratory. Known as the Philly mouse, these mice develop osmotic cataracts by an as yet unknown mechanism.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00119-01 LSR																
PERIOD COVERED October 1, 1981, to September 30, 1982																			
TITLE OF PROJECT (80 characters or less) Central Nervous System Compensation for Peripheral Oculomotor Deficits																			
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																			
<table> <tr> <td>PI: Lance M. Optican</td> <td>Ph.D.</td> <td>Senior Staff Fellow</td> <td>LSR NEI</td> </tr> <tr> <td>Other: David S. Zee</td> <td>M.D.</td> <td>Visiting Scientist</td> <td>CB NEI</td> </tr> <tr> <td></td> <td>M.D.</td> <td>Senior Staff Fellow</td> <td>CB NEI</td> </tr> <tr> <td>Fred C. Chu</td> <td></td> <td></td> <td></td> </tr> </table>				PI: Lance M. Optican	Ph.D.	Senior Staff Fellow	LSR NEI	Other: David S. Zee	M.D.	Visiting Scientist	CB NEI		M.D.	Senior Staff Fellow	CB NEI	Fred C. Chu			
PI: Lance M. Optican	Ph.D.	Senior Staff Fellow	LSR NEI																
Other: David S. Zee	M.D.	Visiting Scientist	CB NEI																
	M.D.	Senior Staff Fellow	CB NEI																
Fred C. Chu																			
COOPERATING UNITS (if any) Department of Neurology, Johns Hopkins University																			
LAB/BRANCH Laboratory of Sensorimotor Research																			
SECTION Oculomotor Control Section																			
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205																			
TOTAL MANYEARS: 1.25	PROFESSIONAL: 1.0	OTHER: 0.25																	
CHECK APPROPRIATE BOX(ES)																			
<input checked="" type="checkbox"/> (a) HUMAN SUBJECTS		<input type="checkbox"/> (b) HUMAN TISSUES																	
<input type="checkbox"/> (a1) MINORS		<input type="checkbox"/> (c) NEITHER																	
SUMMARY OF WORK (200 words or less - underline keywords)																			
<p>Disease or trauma can weaken the extraocular muscles, making it difficult for patients to see clearly. The brain can compensate for these peripheral weaknesses, to some extent, by increasing the innervation sent to the muscles. We studied a patient's adaptation to a lateral rectus palsy when he habitually viewed with the weakened eye. As one expects based on previous publications, the vestibulo-ocular reflex, which moves the eye opposite to head movements, and the rapid, saccadic movements used to change visual fixation all showed adaptive changes. These systems operate in an "open-loop" manner, since they work too fast for visual feedback to have any influence over the individual eye movements. We showed that the pursuit system, which tracks smoothly moving visual targets, also exhibits adaptive increases in innervation. By studying this patient before and after corrective strabismus surgery we were also able to examine the contribution of adaptive mechanisms to the success of the surgical procedures.</p>																			

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00113-02 LSR
PERIOD COVERED October 1, 1981, to September 30, 1982			
TITLE OF PROJECT (80 characters or less) The Neural Coupling between Vergence Eye Movements and Accommodation			
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT			
PI:	Frederick A. Miles D. Phil.	Chief, Oculomotor Control Section	LSR NEI
Other:	Kenji Kawano	M.D., Ph.D. Visiting Scientist	LSR NEI
COOPERATING UNITS (if any) Department of Ophthalmology, The Wilmer Institute of Ophthalmology, Johns Hopkins University School of Medicine			
LAB/BRANCH	Laboratory of Sensorimotor Research		
SECTION	Oculomotor Control Section		
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205			
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:	1.5 1.0 0.5
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER			
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS			
SUMMARY OF WORK (200 words or less - underline keywords) Transfer of fixation between targets at different viewing distances involves changes in <u>vergence eye movements</u> and <u>accommodation</u> that operate to eliminate disparity and blur, respectively. During pinhole viewing, when blur cues are absent, changes in vergence due to disparity result in linear changes in accommodation: the <u>vergence-accommodation response</u> . Experiments were undertaken to determine whether these open-loop responses are subject to visually-mediated <u>adaptive regulation</u> . <u>Human subjects</u> were fitted with specially made laterally-displacing periscopic spectacles to increase the apparent separation of their two eyes and thereby decrease the required change in accommodation per unit change in vergence to maintain single, clear vision. Thirty minutes of exposure to these spectacles was sufficient to cause large decreases in the accommodative change associated with a unit change in vergence during pinhole viewing. This demonstrates that the coupling between vergence and accommodation is subject to adaptive regulation. Decreasing the apparent separation of the eyes with medially-displacing (cyclopean) spectacles failed to affect the magnitude of the vergence-accommodation response. Thus, the adaptive mechanism shows considerable asymmetry.			

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00109-02 LSR
PERIOD COVERED October 1, 1981, to September 30, 1982			
TITLE OF PROJECT (80 characters or less) Visual Motion Processing in the Primate Brain			
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT			
PI:	Robert H. Wurtz	Ph.D.	Chief LSR NEI
Other:	William T. Newsome	Ph.D.	Staff Fellow LSR NEI
	Akichika Mikami	M.D., Ph.D.	Visiting Scientist LSR NEI
COOPERATING UNITS (if any)			
LAB/BRANCH Laboratory of Sensorimotor Research			
SECTION Visuomotor Integration Section			
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205			
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:	
3.0	2.0	1.0	
CHECK APPROPRIATE BOX(ES)			
<input type="checkbox"/> (a) HUMAN SUBJECTS		<input type="checkbox"/> (b) HUMAN TISSUES	
<input checked="" type="checkbox"/> (c) NEITHER			
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS			
SUMMARY OF WORK (200 words or less - underline keywords) <u>Processing of visual information</u> continues beyond the striate cortex in the <u>extrastriate visual areas</u> of the primate cerebral cortex. One of these areas, the <u>middle temporal area</u> , has many neurons that respond selectively to the <u>direction of motion</u> of visual stimuli. We have studied two aspects of cells in this area in <u>awake monkeys</u> able to move their eyes and respond to visual stimuli. The first concerns the way in which these cells respond to the <u>apparent motion</u> of a series of flashed spots of lights that do not move but appear to a human observer to have moved. MT cells show the same directionally selective response to such flashed stimuli as they do to moving stimuli if the frequency of flashes is high enough. This frequency falls into the same range as that producing apparent motion for human observers. The second investigation has shown that areas adjacent to MT have cells that discharge in relation to <u>pursuit eye movements</u> . Histological analysis of myelin stained sections of this region reveals distinct anatomical areas. These experiments indicate that there are a group of prestriate areas probably related to analysis of motion which are probably utilized for both visual perception and oculomotor control.			



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00102-03 LSR
PERIOD COVERED October 1, 1981, to September 30, 1982			
TITLE OF PROJECT (80 characters or less) Role of Substantia Nigra in the Initiation of Eye Movements			
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Robert H. Wurtz Ph.D. Chief LSR NEI Other: Okihide Hikosaka M.D., Ph.D. Visiting Scientist LSR NEI			
COOPERATING UNITS (if any)			
LAB/BRANCH Laboratory of Sensorimotor Research			
SECTION Visuomotor Integration Section			
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205			
TOTAL MANYEARS: 2.5	PROFESSIONAL: 1.5	OTHER: 1.0	
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS			
SUMMARY OF WORK (200 words or less - underline keywords) The <u>basal ganglia</u> of the brain are involved in the initiation of <u>movement</u> . A major output pathway of this structure is the <u>substantia nigra pars reticulata</u> in the <u>brainstem</u> . We have studied cells in this structure, and this report describes two types of neuronal responses that are related to <u>fixation of gaze</u> . Cells with the first type of response decreased their discharge rate following onset of a spot of light in the visual field, but only when the monkey was not looking at another spot of light. This suppression of the visual response was not due to the act of visual fixation but rather to the presence of the visual stimulus during fixation. The point in the visual field that gave the most vigorous suppression of the high background rate was always located near the fovea. The second type of response was to the offset of the spot of light on which the monkey was fixating; the presence of any spot of light, other than the fixation light, in the visual field reduced this response. Response of these two types of cells seems to occur only at the initiation or the termination of a series of visually-guided saccades and might signal the transition between <u>spontaneous saccades</u> and <u>visually-guided saccades</u> .			



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EX 00055-04 LSR
PERIOD COVERED October 1, 1981, to September 30, 1982			
TITLE OF PROJECT (80 characters or less) Visual and Oculomotor Functions of the Primate Superior Colliculus			
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Joanne E. Albano Ph.D. Staff Fellow LSR NEI Other: George F. Creswell B.S. Histologist LSR NEI			
COOPERATING UNITS (if any)			
LAB/BRANCH Laboratory of Sensorimotor Research			
SECTION Visuomotor Integration Section			
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205			
TOTAL MANYEARS: 1.5	PROFESSIONAL: 1.0	OTHER: 0.5	
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS			
SUMMARY OF WORK (200 words or less - underline keywords) Pathways providing potential <u>sources of saccadic input</u> to the <u>primate superior colliculus</u> were investigated using <u>neuroanatomical tracing</u> techniques. Injections of horseradish peroxidase, a retrograde tracer, were made at various depths within the superior colliculus, including the stratum griseum intermediale, where electrical stimulation elicits saccadic eye movements with low thresholds. Other injections were made above and below this low threshold region. We found that numerous, diverse regions of the brainstem and frontal cortex project to the deep layers of the superior colliculus and subjacent mesencephalic reticular formation. However, sources of subcortical and frontal-cortical input to the intermediate layers of the colliculus are more limited than previously thought. These experiments indicate that three subcortical structures, the substantia nigra, the parabigeminal nucleus, and the rostral mesencephalic reticular formation, and a subregion of prearcuate cortex known as the frontal eye fields may provide the most prominent inputs to the presaccadic cells of the intermediate layers.			



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER
			Z01 EY 00049-04 LSR
PERIOD COVERED			
October 1, 1981, to September 30, 1982			
TITLE OF PROJECT (80 characters or less)			
Cerebral Cortical Mechanisms for Eye Movements and Visual Attention			
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT			
<p>PI: Michael E. Goldberg M.D. Chief, Section on LSR NEI Neuro-Ophthalmologic Mechanisms</p> <p>Other: Charles J. Bruce Ph.D. Senior Staff Fellow LSR NEI</p>			
COOPERATING UNITS (if any)			
<p>Department of Neurology, Georgetown University School of Medicine</p> <p>Department of Anatomy, Howard University</p>			
LAB/BRANCH			
Laboratory of Sensorimotor Research			
SECTION			
Neuro-Ophthalmologic Mechanisms Section			
INSTITUTE AND LOCATION			
National Eye Institute, NIH, Bethesda, Maryland 20205			
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:	
2.5	1.5	1.0	
CHECK APPROPRIATE BOX(ES)			
<input type="checkbox"/> (a) HUMAN SUBJECTS		<input type="checkbox"/> (b) HUMAN TISSUES	
		<input checked="" type="checkbox"/> (c) NEITHER	
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS			
SUMMARY OF WORK (200 words or less - underline keywords)			
<p>Studies are being conducted to determine the mechanisms through which the <u>frontal eye fields</u> of the <u>cerebral cortex</u> exert control over <u>eye movements</u> and <u>visual attention</u> in the monkey. <u>Single-cell recordings</u> are made while the monkeys perform a series of visual tasks involving eye movements or visual fixation. Previous work in this laboratory has found a neural mechanism for the generation of visually-guided eye movements in frontal eye fields. The frontal eye fields have a topographic map, so that cells associated with large eye movements are located dorsomedially in the frontal eye fields, and cells subserving small eye movements are located ventrolaterally. Cells in the area subserving the largest eye movements have auditory as well as or instead of visual responses. Cells near the region subserving small saccades discharge during smooth pursuit eye movements, and electrical stimulation here evokes smooth eye movements. The frontal eye fields have a topographic projection to the area of the superior colliculus subserving eye movements, so that the area related to large eye movements projects to the caudal superior colliculus, and the area related to small eye movements projects to the rostral superior colliculus.</p>			



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00045-04 LSR								
PERIOD COVERED October 1, 1981, to September 30, 1982											
TITLE OF PROJECT (80 characters or less) Visuomotor Properties of Neurons in the Thalamus											
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT											
<table> <tr> <td>PI: David Lee Robinson</td> <td>Ph.D. Research Physiologist</td> <td>LSR</td> <td>NEI</td> </tr> <tr> <td>Other: Steven E. Petersen</td> <td>Ph.D. Staff Fellow</td> <td>LSR</td> <td>NEI</td> </tr> </table>				PI: David Lee Robinson	Ph.D. Research Physiologist	LSR	NEI	Other: Steven E. Petersen	Ph.D. Staff Fellow	LSR	NEI
PI: David Lee Robinson	Ph.D. Research Physiologist	LSR	NEI								
Other: Steven E. Petersen	Ph.D. Staff Fellow	LSR	NEI								
COOPERATING UNITS (if any)											
LAB/BRANCH Laboratory of Sensorimotor Research											
SECTION Visuomotor Integration Section											
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205											
TOTAL MANYEARS: 3.0	PROFESSIONAL: 2.0	OTHER: 1.0									
CHECK APPROPRIATE BOX(ES)											
<input type="checkbox"/> (a) HUMAN SUBJECTS		<input type="checkbox"/> (b) HUMAN TISSUES									
		<input checked="" type="checkbox"/> (c) NEITHER									
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS											
SUMMARY OF WORK (200 words or less - underline keywords) Four subdivisions of the <u>pulvinar</u> have been studied in awake monkeys to determine their contribution to <u>vision</u> and <u>visual behavior</u> . The beta portion of the <u>lateral pulvinar</u> contains a population of cells with <u>saccade-related activity</u> . Such cells discharge with eye movements, even when made spontaneously in the dark. Other cells are visual; most responses to stimulus movement are <u>pan-directional</u> (equal activity to all directions) while a few are <u>directionally-selective</u> (activity for some but not all directions). The mean latency is long and the standard deviation is large; these are also characteristic of its cortical target, area 7. The <u>inferior pulvinar</u> and adjacent alpha division of the lateral pulvinar are similar; both have a concentration of visual cells, with pan-directionality and directional selectivity common. The mean and standard deviation of latencies are different from the beta subdivision. The few cells with saccade-related activity are visually responsive and their eye movement activity is visually mediated. The <u>medial pulvinar</u> is a hybrid of the other pulvinar nuclei. There are functional differences between pulvinar subdivisions which correlate with their cortical target areas.											

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00047-04 LSR																								
PERIOD COVERED October 1, 1981, to September 30, 1982																											
TITLE OF PROJECT (80 characters or less) Visual Processing in Brains following Cortical Ablation																											
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																											
<table> <tr> <td>PI:</td> <td>Michael E. Goldberg</td> <td>M.D.</td> <td>Chief, Section on Neuro-Ophthalmologic Mechanisms</td> <td>LSR</td> <td>NEI</td> </tr> <tr> <td>Other:</td> <td>Charles J. Bruce</td> <td>Ph.D.</td> <td>Senior Staff Fellow</td> <td>LSR</td> <td>NEI</td> </tr> <tr> <td></td> <td>Leslie G. Ungerleider</td> <td>Ph.D.</td> <td>Senior Staff Fellow</td> <td>LN</td> <td>NIMH</td> </tr> <tr> <td></td> <td>Mortimer Mishkin</td> <td>Ph.D.</td> <td>Research Physiologist</td> <td>LN</td> <td>NIMH</td> </tr> </table>				PI:	Michael E. Goldberg	M.D.	Chief, Section on Neuro-Ophthalmologic Mechanisms	LSR	NEI	Other:	Charles J. Bruce	Ph.D.	Senior Staff Fellow	LSR	NEI		Leslie G. Ungerleider	Ph.D.	Senior Staff Fellow	LN	NIMH		Mortimer Mishkin	Ph.D.	Research Physiologist	LN	NIMH
PI:	Michael E. Goldberg	M.D.	Chief, Section on Neuro-Ophthalmologic Mechanisms	LSR	NEI																						
Other:	Charles J. Bruce	Ph.D.	Senior Staff Fellow	LSR	NEI																						
	Leslie G. Ungerleider	Ph.D.	Senior Staff Fellow	LN	NIMH																						
	Mortimer Mishkin	Ph.D.	Research Physiologist	LN	NIMH																						
COOPERATING UNITS (if any) Department of Neurology, Georgetown University School of Medicine Laboratory of Neuropsychology, NIMH																											
LAB/BRANCH Laboratory of Sensorimotor Research																											
SECTION Neuro-Ophthalmologic Mechanisms Section																											
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205																											
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:																									
0.5	0.5	0.0																									
CHECK APPROPRIATE BOX(ES)																											
<input type="checkbox"/> (a) HUMAN SUBJECTS		<input type="checkbox"/> (b) HUMAN TISSUES		<input checked="" type="checkbox"/> (c) NEITHER																							
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																											
<p>SUMMARY OF WORK (200 words or less - underline keywords)</p> <p>The <u>striate cortex</u> of one <u>cerebral hemisphere</u> of a <u>rhesus monkey</u> is removed surgically under direct vision. The monkeys are allowed to recover from the effects of surgery in a normally lit environment. The monkeys are then trained on a series of tasks requiring <u>visual perception</u> and <u>visually-guided eye movements</u>. They are then prepared for chronic neurophysiological recording and for eye position recording. The activity of <u>single neurons</u> in the <u>frontal eye fields</u> and <u>posterior parietal cortex</u> both ipsilateral and contralateral to the lesion is studied. The monkey's oculomotor capacity is studied quantitatively. The frontal eye fields are much less visually responsive than normal although eye movements can still be evoked at low threshold from the silent area. The animals can make normal <u>saccadic eye movements</u> to stationary targets in the blind field although they cannot make accurate saccades to moving stimuli. They cannot use stimulus velocity or position information in the contralateral field to generate a <u>smooth pursuit eye movement</u>. These data indicate that the striate cortex is necessary for the normal functioning of visual association areas, including those involved in oculomotor processing.</p>																											



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00120-02 CB
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PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Laser Instrumentation for Vitreous Surgery

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Sanford M. Meyers	M.D.	Senior Staff Ophthalmologist	CB	NEI
Other: Robert F. Bonner	Ph.D.	Physicist	BEIB	DRS
Stephen B. Leighton	Ph.D.	Engineer	BEIB	DRS
Merlyn M. Rodrigues	M.D.	Chief, Section on Clinical Eye Pathology	CB	NEI

COOPERATING UNITS (if any)

Division of Research Services Biomedical Engineering Instrumentation Branch

LAB/BRANCH

Clinical Branch

SECTION

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.85	0.60	0.25

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A carbon dioxide laser with a special delivery system for use in vitreous surgery has been developed and is undergoing testing in animals. Preliminary data reveal that the carbon dioxide laser instrument appears beneficial in certain aspects of vitreous surgery.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00057-04 CB
PERIOD COVERED October 1, 1981, to September 30, 1982			
TITLE OF PROJECT (80 characters or less) Ocular Connective Tissue Macromolecules and Their Function in Vision			
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT			
PI: Pamela Gehron Robey	Ph.D.	Staff Fellow	CB NEI
Other: David A. Newsome	M.D.	Chief, Section on Retinal and Ocular Connective Tissue Diseases	CB NEI
Judy A. Kirshner	B.S.	Biological Aide	CB NEI
COOPERATING UNITS (if any)			
Laboratory of Developmental Biology and Anomalies, NIDR			
LAB/BRANCH Clinical Branch			
SECTION Section on Retinal and Ocular Connective Tissue Diseases			
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205			
TOTAL MANYEARS: 1.50	PROFESSIONAL: 1.25	OTHER: 0.25	
CHECK APPROPRIATE BOX(ES)			
<input type="checkbox"/> (a) HUMAN SUBJECTS		<input checked="" type="checkbox"/> (b) HUMAN TISSUES	<input type="checkbox"/> (c) NEITHER
<input type="checkbox"/> (a1) MINORS		<input type="checkbox"/> (a2) INTERVIEWS	
SUMMARY OF WORK (200 words or less - underline keywords)			
<u>Extra cellular matrix</u> components, such as <u>collagen</u> , <u>proteoglycans</u> and other <u>glycoconjugates</u> are being identified in ocular tissues such as <u>trabecular meshwork</u> , <u>sclera</u> , <u>lens capsule</u> and <u>chorioretinal complex</u> , with particular emphasis on <u>Bruch's membrane</u> . These components are isolated from the tissues by various extraction procedures and compared to the components synthesized by <u>organ culture</u> of these tissues in the presence of radiolabeled precursors. <u>Biochemical characterization</u> is accomplished by column chromatography, sodium dodecyl sulfate polyacrylamide gel electrophoresis and enzymatic and chemical degradations. Alterations of the connective tissue components may play a role in certain ocular diseases such as in <u>glaucoma</u> and in <u>drusenoid macular degeneration</u> . Diseased tissues are also being studied to gain insight into the role of extracellular matrix components in these disorders.			

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00122-02 CB
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PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Anatomical Studies of the Visual System of Primates

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Stanley J. Schein	M.D., Ph.D.	Expert	CB	NEI
Other: Francisco de Monasterio	M.D., D.Sc.	Chief, Section on Visual Processing	CB	NEI
Rafael C. Caruso	M.D.	Expert	CB	NEI
Edna P. McCrane	B.S.	Biologist	CB	NEI
Marvin B. Shapiro	M.A.	Research Mathematician	LSMM	DCRT
Jim E. Fullbrook	Ph.D.	Guest Worker	CB	NEI
J. Kelly Newlander		Summer Student	CB	NEI
Jorge A. Martinez	B.S.	Technician	CB	NEI

COOPERATING UNITS (if any)

Laboratory of Statistical and Mathematical Methodology, DCRT

LAB/BRANCH

Clinical Branch

SECTION

Section on Visual Processing

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 5.0	PROFESSIONAL: 5.0	OTHER: 0.0
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CHECK APPROPRIATE BOX(Es)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The aim of this project is to study the functional anatomical organization of neurons of the visual system of non-human primates that can serve as a model for the human visual system. Parcellation of the monkey visual cortex is based on silver cell, silver myelin and cytochrome oxidase staining and on-connectional studies. Cytochrome oxidase staining is also being used to activity label chronic stimulation states in the brain, and improvements in the 2-deoxy-glucose activity labelling method for acute stimulation states has been developed. Further improvements are in progress. Retinal studies have focused on blue-sensitive cones, their pattern, synaptology, and the mechanism by which blue-sensitive cones may be specifically stained.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00063-04 CB
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PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Acquired and Congenital Color Vision Deficiencies: Mechanisms and Diagnosis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Francisco M. de Monasterio	M.D., D.Sc.	Chief, Section on Visual Processing	CB	NEI
Other:	Kent E. Higgins	Ph.D.	Senior Staff Fellow	CB	NEI
	Stanley J. Schein	M.D., Ph.D.	Expert	CB	NEI
	Rafael C. Caruso	M.D.	Expert	CB	NEI
	Myles J. Jaffe	O.D.	Guest Worker	CB	NEI
	Marvin J. Podgor	M.S.	Statistician	OBE	NEI
	Edna P. McCrane	B.S.	Biologist	CB	NEI
	Patricia Mercer	B.S.	Health Technician	CB	NEI
	Doris Collie		Health Technician	CB	NEI
	Mary Fuhrman		Health Technician	CB	NEI
	J. Kelly Newlander		Summer Student	CB	NEI

COOPERATING UNITS (if any)

Lion's Eye Bank, Washington, D.C.

LAB/BRANCH

Clinical Branch

SECTION

Section on Visual Processing

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.0	6.0	0.0

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is a study of cone function in selected cases of color vision defects, with special emphasis on acquired defects. Human subjects are examined with electrophysiological and psychophysical tests, while experimental studies are carried out in subhuman primates.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00059-04 CB
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PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Electrophysiological and Psychophysical Evaluation of Retinal Disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Francisco M. de Monasterio	M.D., D.Sc.	Chief, Section on Visual Processing	CB	NEI
Other:	Kent E. Higgins	Ph.D.	Senior Staff Fellow	CB	NEI
	Rafael C. Caruso	M.D.	Expert	CB	NEI
	Ralph D. Gunkel	O.D.	Ophthalmic Physicist	CB	NEI
	Myles J. Jaffe	O.D.	Guest Worker	CB	NEI
	Stanley J. Schein	M.D., Ph.D.	Expert	CB	NEI
	Doris Collie	A.A.	Health Technician	CB	NEI
	Mary Fuhrman		Health Technician	CB	NEI
	Patricia Mercer	B.S.	Health Technician	CB	NEI

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Branch

SECTION

Section on Visual Processing

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.75	6.75	0.0

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This is a general support and service-providing project for the inflammatory, degenerative, or congenital retinal disorders, and to conduct tests and experiments directed towards the clinical application and development of electrophysiological and psychophysical procedures for measuring visual function in patients of NEI's Eye Clinic and of other services in the NIH Clinical Center.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00065-05 CB
PERIOD COVERED October 1, 1981, to September 30, 1982			
TITLE OF PROJECT (80 characters or less) Physiological Studies of the Visual System of Primates			
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT			
PI:	Francisco M. de Monasterio M.D., D.Sc.	Chief, Section on Visual Processing	CB NEI
Other:	Stanley J. Schein M.D., Ph.D.	Expert	CB NEI
	Edna P. McCrane B.S.	Biologist	CB NEI
	Kent E. Higgins Ph.D.	Senior Staff Fellow	CB NEI
	Robert Desimone Ph.D.	Staff Fellow	LN NIMH
COOPERATING UNITS (if any)			
LAB/BRANCH Clinical Branch			
SECTION Section on Visual Processing			
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205			
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:	
3.5	3.5	0.0	
CHECK APPROPRIATE BOX(ES)			
<input type="checkbox"/> (a) HUMAN SUBJECTS		<input type="checkbox"/> (b) HUMAN TISSUES	
		<input checked="" type="checkbox"/> (c) NEITHER	
<input type="checkbox"/> (a1) MINORS		<input type="checkbox"/> (a2) INTERVIEWS	
SUMMARY OF WORK (200 words or less - underline keywords)			
<p>This project is a study of the physiological organization of neurons in the visual system of non-human primates that may serve as a model for the human visual system. The project gives emphasis to the <u>chromatic</u> and <u>spatial</u> <u>properties</u> and <u>central projections</u> of neurons of the <u>retina</u>, <u>lateral geniculate body</u>, <u>striate cortex</u> and <u>extrastriate cortex</u> of macaque monkeys.</p>			

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00006-11 CB
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PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Research in Methods of Evaluating Visual Processes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Ralph D. Gunkel	O.D.	Ophthalmic Physicist	CB	NEI
Other: David G. Cogan	M.D.	Medical Officer	CB	NEI
	M.D.	Senior Staff Fellow	CB	NEI
Fred C. Chu				
David A. Newsome	M.D.	Senior Staff Fellow	CB	NEI

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Branch

SECTION

Section on Visual Processing

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Appropriate psychophysical procedures are used to measure the various visual functions of patients in the Eye Clinic, particularly thresholds of visibility for the retinal rods and cones and for discrimination of colors, all under standard conditions. These tests are done for the purpose of discovering and monitoring any changes in visual efficiency due to degenerative diseases or toxic medications. Efforts continue in attempts to find or devise test procedures which are more effective, more objective, and less demanding on the patients.

Tests were conducted on 377 patients during the past year.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00061-04 CB
PERIOD COVERED October 1, 1981, to September 30, 1982			
TITLE OF PROJECT (80 characters or less) Retinal Function in Posterior Uveitis			
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT			
PI:	Francisco M. de Monasterio	M.D., D.Sc. Chief, Section on Visual Processing	CB NEI
Other:	Kent E Higgins	Ph.D. Senior Staff Fellow	CB NEI
	Stanley J. Schein	M.D., Ph.D. Expert	CB NEI
	Myles J. Jaffe	O.D. Guest Worker	CB NEI
	Patricia Mercer	B.S. Health Technician	CB NEI
	Doris Collie	Health Technician	CB NEI
	Mary Fuhrman	Health Technician	CB NEI
COOPERATING UNITS (if any)			
LAB/BRANCH Clinical Branch			
SECTION Section on Visual Processing			
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205			
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:	
2.7	2.7	0.0	
CHECK APPROPRIATE BOX(ES)			
<input checked="" type="checkbox"/> (a) HUMAN SUBJECTS		<input type="checkbox"/> (b) HUMAN TISSUES	
		<input type="checkbox"/> (c) NEITHER	
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS			
SUMMARY OF WORK (200 words or less - underline keywords)			
<p>Abnormalities of retinal function at the level of <u>rods and cones</u> or their <u>pathways</u> are being documented by <u>electrophysiological</u> and <u>psychophysical</u> <u>studies of patients with posterior uveitis</u> of suspected <u>immunological origin</u>, in addition to experimental studies of posterior uveitis in animal models.</p>			



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00121-02 CB
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PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Spatial Contrast Sensitivity Studies in Retinal and Neuro-ophthalmological Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Kent E. Higgins	Ph.D.	Senior Staff Fellow	CB	NEI
Other: Francisco M. de Monasterio	M.D., D.Sc.	Chief, Section on Visual Processing	CB	NEI
Myles J. Jaffe	O.D.	Guest Worker	CB	NEI
Rafael C. Caruso	M.D.	Expert	CB	NEI
Brooke Shefrin	B.S.	Guest Worker	CB	NEI

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Branch

SECTION

Section on Visual Processing

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 2.3	PROFESSIONAL: 2.3	OTHER: 0.0
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CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to provide diagnosis assessment of deficits and alterations in visual resolution in toxic, inflammatory, degenerative, or congenital retinal and neuro-ophthalmological disorders through the measurement of overall spatial contrast sensitivity.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00123-02 CB
PERIOD COVERED <u>October 1, 1981 to September 30, 1982</u>			
TITLE OF PROJECT (80 characters or less) <u>Psychophysical Studies in Hemianopia</u>			
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT			
PI:	Rafael C. Caruso	M.D.	Expert CB NEI
Other:	Myles J. Jaffe	O.D.	Guest Worker CB NEI
	Kent E. Higgins	Ph.D.	Senior Staff Fellow CB NEI
	Francisco de Monasterio	M.D., D. Sc.	Chief, Section on CB NEI Visual Processing
	Patricia Mercer	B.S.	Health Technician CB NEI
	Doris Collie	A.A.	Health Technician CB NEI
	Mary Fuhrman		Health Technician CB NEI
COOPERATING UNITS (if any) Department of Radiology, CC Branch of Developmental Endocrinology, NICHD			
LAB/BRANCH Clinical Branch			
SECTION Section on Visual Processing			
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205			
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:	
1.0	1.0	0.0	
CHECK APPROPRIATE BOX(ES)			
<input checked="" type="checkbox"/> (a) HUMAN SUBJECTS		<input type="checkbox"/> (b) HUMAN TISSUES	
		<input type="checkbox"/> (c) NEITHER	
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS			
SUMMARY OF WORK (200 words or less - underline keywords)			
<p>The visual function of patients with <u>chiasmatic</u> and <u>retrochiasmatic</u> <u>lesions</u> of the visual pathways is assessed with <u>psychophysical</u> tests. These include <u>kinetic</u> and <u>static perimetry</u>, <u>color vision</u> tests and <u>spatial contrast sensitivity</u> studies. The purpose of this study is to identify and develop tests to characterize the nature and evolution of <u>visual loss</u> in lesions that cause hemianopia.</p>			



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00144-01 CB
PERIOD COVERED October 1, 1981, to September 30, 1982			
TITLE OF PROJECT (80 characters or less) Visual Evoked Responses in Lesions of the Visual Pathways			
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT			
PI:	Rafael C. Caruso	M.D.	Expert CB NEI
Other:	Lezheng Wu	M.D.	Visiting Fellow CB NEI
	Francisco M. de Monasterio	M.D., D.Sc.	Chief, Section on CB NEI Visual Processing
	Myles J. Jaffe	O.D.	Guest Worker CB NEI
	Patricia Mercer	M.S.	Health Technician CB NEI
	Doris Collie	A.A.	Health Technician CB NEI
	Mary Fuhrman		Health Technician CB NEI
COOPERATING UNITS (if any)			
LAB/BRANCH Clinical Branch			
SECTION Section on Visual Processing			
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205			
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:	
2.0	2.0	0.0	
CHECK APPROPRIATE BOX(ES)			
<input checked="" type="checkbox"/> (a) HUMAN SUBJECTS		<input type="checkbox"/> (b) HUMAN TISSUES	
		<input type="checkbox"/> (c) NEITHER	
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS			
SUMMARY OF WORK (200 words or less - underline keywords)			
<p>Visual evoked responses are recorded in <u>normal volunteers</u> and in <u>patients with lesions of the retina, optic nerves, optic chiasm, optic radiations and visual cortex</u>. Both pattern stimuli and unstructured stimuli are used. The recordings are used for <u>diagnostic</u> purposes and to provide an <u>objective assessment</u> of visual function in these conditions. These data are correlated with the results of <u>psychophysical tests of visual function</u>.</p>			



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00135-10 CB
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PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Biochemistry of Retina and Pigmented Epithelium in Health and Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Helen H. Hess,	M.D. Medical Officer	CB NEI
Other: David A. Newsome	M.D. Chief, Section on Retinal and Ocular Connective Tissue Diseases	CB NEI
Gloria E. Westney	B.S. Biologist	CB NEI
Joseph J. Knapka	Ph.D. Nutritionist, Small Animal Section	VRB DRS
Ira Levine	B.S. Guest Worker	CB NEI

COOPERATING UNITS (if any)

Veterinary Resources Branch, DRS, NIH

LAB/BRANCH

Clinical Branch

SECTION

Section on Retinal and Ocular Connective Tissue Diseases

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.8	1.3	0.5

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Investigations are being conducted into the biochemical composition of the sensory retina, pigmented epithelium, choroid and biological fluids in normal and disease states, particularly in animal models of human retinal degenerations and in human retinal diseases. The tissue specific distributions of inorganic constituents are studied by flameless atomic absorption, with concentrations of Cu, Zn and Ca of particular interest. These elements are being assayed in 24 hour urine specimens from patients with retinal degenerations (retinitis pigmentosa and macular degenerations). The effects of nutrition and genetic background on the progress of chorioretinal degeneration in the retinal dystrophic pigmented RCS rat are being investigated. Age of onset and incidence of posterior subcapsular cataracts and the history of progression of mature cataracts are being studied in both pink-eyed and black-eyed retinal dystrophic RCS rat models of retinal degeneration-related cataract. Nutritional and environmental factors involved in incidence and prevention of the cataracts are being investigated.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00030-11 CB
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PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Studies of Parameters of Intraocular Pressure

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Douglas E. Gaasterland	M.D.	Chief, Section on Glaucoma	CB	NEI
Other:	Carl Kupfer	M.D.	Director	NEI	
	Lessie McCain	R.N.	Clinical Technician	CB	NEI
	Roy Milton	Ph.D.	Chief, Section on Biometry	OBE	NEI
	Elmer J. Ballantine	M.D.	Clinical Director	CB	NEI

COOPERATING UNITS (if any)

Pharmaceutical Development Service, CC, NIH
Department of Ophthalmology, Mayo Clinic, Rochester, Minnesota

LAB/BRANCH

Clinical Branch

SECTION

Section on Glaucoma

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.10	0.05	0.05

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Young and old normal volunteers and patients with glaucoma or ocular hypertension participate in this continuing study of the parameters of intraocular pressure. The acute and long-term effects of antiglaucoma medications alone and in combination upon the parameters are studied in normal and in diseased eyes.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00050-06 CB
PERIOD COVERED October 1, 1981, to September 30, 1982			
TITLE OF PROJECT (80 characters or less) Aqueous Humor Flow Measurement by Fluorophotometry			
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT			
PI:	Douglas E. Gaasterland M.D. Chief, Section on Glaucoma	CB	NEI
Other:	Lessie McCain R.N. Clinical Technician	CB	NEI
COOPERATING UNITS (if any)			
LAB/BRANCH Clinical Branch			
SECTION Section on Glaucoma			
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205			
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:	
0.01	0.01	0.00	
CHECK APPROPRIATE BOX(ES)			
<input checked="" type="checkbox"/> (a) HUMAN SUBJECTS		<input type="checkbox"/> (b) HUMAN TISSUES	
		<input type="checkbox"/> (c) NEITHER	
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS			
SUMMARY OF WORK (200 words or less - underline keywords)			
<p>The <u>aqueous humor flow</u> in humans is measured by determining the rate of loss of <u>fluorescein</u> from the eye after <u>iontophoresis</u> into the cornea in <u>normal volunteers</u> and in <u>patients with ocular hypertension or glaucoma</u>.</p>			



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00154-09 CB
PERIOD COVERED October 1, 1981, to September 30, 1982			
TITLE OF PROJECT (80 characters or less) Experimental Glaucoma in the Rhesus Monkey			
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Douglas E. Gaasterland M.D. Chief, Section on Glaucoma CB NEI Other: Merlyn Rodrigues M.D. Chief, Section on Clinical CB NEI Eye Pathology			
COOPERATING UNITS (if any)			
LAB/BRANCH Clinical Branch			
SECTION Section on Glaucoma			
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205			
TOTAL MANYEARS: 0.02	PROFESSIONAL: 0.02	OTHER: 0.0	
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS			
SUMMARY OF WORK (200 words or less - underline keywords) <p>The purpose of this investigation is to study the <u>morphology</u>, <u>physiologic function</u>, and <u>pharmacologic responses</u> in the eye of the rhesus monkey in its <u>normal state</u> compared to its state when <u>experimental glaucoma</u> has been induced by <u>argon laser photocoagulation</u> of the <u>trabecular meshwork</u>.</p>			



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00046-06 CB
PERIOD COVERED October 1, 1981, to September 30, 1982		
TITLE OF PROJECT (80 characters or less) Laboratory Studies of Aqueous Humor Dynamics		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Douglas E. Gaasterland M.D.	Chief, Section on Glaucoma	CB NEI
Other: John A. Barranger M.D.	Chief, Clinical Section	DMNB NINCDS
Toichiro Kuwabara M.D.	Chief, Section on Experimental Pathology	LVR NEI
Pamela Robey Ph.D.	Staff Fellow	CB NEI
Claude C. Cummins, III BS	Summer Student (Biologist)	CB NEI
COOPERATING UNITS (if any) Developmental and Metabolic Neurology Branch, NINCDS, NIH		
LAB/BRANCH Clinical Branch		
SECTION Section on Glaucoma		
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0.05	PROFESSIONAL: 0.05	OTHER: 0.0
CHECK APPROPRIATE BOX(ES)		
<input type="checkbox"/> (a) HUMAN SUBJECTS	<input type="checkbox"/> (b) HUMAN TISSUES	<input checked="" type="checkbox"/> (c) NEITHER
<input type="checkbox"/> (e1) MINORS	<input type="checkbox"/> (e2) INTERVIEWS	
SUMMARY OF WORK (200 words or less - underline keywords)		
<p>Investigations have been done to clarify <u>intraocular fluid movement in rhesus monkeys and humans</u>. A method was perfected for spectrophotometric determinations of <u>ascorbic acid concentration</u> in ocular and systemic fluids. This is being applied to human aqueous samples. Monkey <u>aqueous humor</u> has been analyzed for <u>glycosaminoglycan</u>, <u>glycoprotein</u> and <u>hyaluronidase</u> content.</p>		



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER
		Z01 EY 00168-07 CB

PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Laser Surgery for Glaucoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Douglas E. Gaasterland M.D.	Chief, Section on Glaucoma	CB	NEI
Other:	Charles Bonney	D.V.M., Ph.D. Visiting Scientist	CB	NEI
	Elmer J. Ballantine	M.D. Clinical Director	CB	NEI
	Robert Bonner	Ph.D. Physicist	BEIB	DRS
	Claude Cummins	B.S. Biologist	CB	NEI
	Alan H. Rich	B.S. Engineer	BEIB	DRS
	Sumana K. Davi	Ph.D. Expert	CB	NEI
	Gerald W. Liesegang	Ph.D. Senior Staff Fellow	IR-TD	NHLBI
	Merlyn Rodrigues	M.D., Ph.D. Chief, Section on Clinical Eye Pathology	CB	NEI

COOPERATING UNITS (if any)

Biomedical Engineering and Instrumentation Branch, DRS; Armed Forces
Radiobiology Research Institute

LAB/BRANCH

Clinical Branch

SECTION

Section on Glaucoma

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.31	1.21	0.1

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The high energy and power of lasers offer a tool for noninvasive alteration of anterior intraocular tissue. Specifically, iridotomy and trabeculotomy are possible. This has importance for glaucoma patients because of the potential improvement of surgical outcome and reduced surgical morbidity. The aim of this project is a systematic evaluation of laser effects in simian (rhesus) eyes and the application of promising systems and procedures to human glaucoma eyes under controlled conditions.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00077-05 CB
PERIOD COVERED October 1, 1981, to September 30, 1982			
TITLE OF PROJECT (80 characters or less) Treatment of Neovascular Glaucoma			
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Douglas E. Gaasterland M.D. Chief, Section on Glaucoma CB NEI Other: Elmer J. Ballantine M.D. Clinical Director CB NEI			
COOPERATING UNITS (if any)			
LAB/BRANCH Clinical Branch			
SECTION Section on Glaucoma			
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205			
TOTAL MANYEARS: 0.00	PROFESSIONAL: 0.00	OTHER: 0.0	
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS			
SUMMARY OF WORK (200 words or less - underline keywords) Patients with <u>rubeosis iridis</u> and <u>neovascular glaucoma</u> are being recruited. Those with salvageable vision are invited to join this prospective, randomized study of whether cyclocryotherapy or cyclodiathermy is better for the treatment of this disease. Outcome will be judged by assessing preservation of <u>visual function</u> ; adequate control of <u>intraocular pressure</u> , with or without medications; and control of <u>discomfort</u> . It is estimated that approximately 40 nondiabetic and 40 diabetic patients are needed for this project.			

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00143-09 CB
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PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Radioiodinated Chloroquine Analog for Diagnosis of Ocular Melanoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Douglas E. Gaasterland M.D.	Chief, Section on Glaucoma	CB	NEI
Other:	Elmer J. Ballantine M.D.	Clinical Director	CB	NEI
	Carl Kupfer M.D.	Director		NEI

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Branch

SECTION

Section on Glaucoma

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0.02	PROFESSIONAL: 0.02	OTHER: 0.0
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CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Thirty-six patients with pigmented ocular lesions originally participated in this study. The early results of the study show that the diagnostic technique had inadequate specificity. For most patients a clear clinical diagnosis has been made, and their ocular problem resolved. Except for occasional follow-up examinations of some of the patients, work on this project has ended.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER
		Z01 EY 00022-08 CB

PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Urokinase Central Retinal Vein Occlusion Trial

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Elmer J. Ballantine	M.D.	Clinical Director	CB	NEI
Other:	Harvey R. Gralnick	M.D.	Chief, Hematology Service	CC	NIH
	Daniel G. Seigel	Sc.D.	Deputy Chief, Office of Biometry	OBE	NEI

COOPERATING UNITS (if any)

Office of Biometry and Epidemiology, NEI

LAB/BRANCH

Clinical Branch

SECTION

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Patients with recent complete occlusion of the central retinal vein are randomly assigned to treatment either with intravenous urokinase followed by heparin, heparin alone or intravenous fluids alone. The patients are then examined periodically for one year, and the effectiveness of treatment is judged by restoration of vision and the degree of protection achieved against the development of hemorrhagic glaucoma.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00150-09 CB
PERIOD COVERED <u>October 1, 1981, to September 30, 1982</u>			
TITLE OF PROJECT (80 characters or less) Ocular Hypertension Study			
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT			
PI: Elmer J. Ballantine M.D. Clinical Director CB NEI Other: Douglas E. Gaasterland M.D. Chief, Section on Glaucoma CB NEI Richard Weiblinger B.S. Biologist CB NEI			
COOPERATING UNITS (if any) Office of Biometry and Epidemiology, NEI			
LAB/BRANCH Clinical Branch			
SECTION			
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205			
TOTAL MANYEARS: 1.0	PROFESSIONAL: 0.6	OTHER: 0.4	
CHECK APPROPRIATE BOX(ES)			
<input checked="" type="checkbox"/> (a) HUMAN SUBJECTS		<input type="checkbox"/> (b) HUMAN TISSUES	
		<input type="checkbox"/> (c) NEITHER	
<input type="checkbox"/> (a1) MINORS		<input type="checkbox"/> (a2) INTERVIEWS	
SUMMARY OF WORK (200 words or less - underline keywords)			
<p>Patients with <u>ocular hypertension</u> are randomly assigned to treatment with topical <u>pilocarpine</u> in one or both eyes or to no treatment. The objectives of the study are: 1) to determine if treatment with pilocarpine to reduce intraocular pressure before visual field changes occur will reduce the number of ocular hypertensive subjects who eventually become glaucomatous, and 2) to determine if measurements of aqueous humor dynamics, the response to water loading of diurnal variation in intraocular pressure, serial stereophotographs of the optic disc, and measurements of visual fields help to predict which patients will eventually become glaucomatous.</p>			



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00107-03 CB
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PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Suppression of Retinoblastoma Proliferation by Human Soluble Lymphocyte Factors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Mario Salinas-Carmona	M.D.	Visiting Fellow	CB	NEI
Other: Robert Nussenblatt	M.D.	Chief, Ophthalmic Immunology Section	CB	NEI
Paul Russell	Ph.D.	Research Chemist	LVR	NEI
John Hooks	Ph.D.	Research Microbiologist	LOM	NIDR

COOPERATING UNITS (if any)

Laboratory of Oral Medicine, NIDR

LAB/BRANCH
Clinical BranchSECTION
Section on Ophthalmic ImmunologyINSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.0	PROFESSIONAL: 0.25	OTHER: 0.75
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CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Mononuclear cells when stimulated with concanavalin A develop different biological activities, including suppressor activity. The objective of this work is to find out whether the inhibitory activity is mediated through soluble factors, and to characterize these factors' biological and physiochemical properties. We have found that the factors responsible for suppression are non-dialyzable, heat stable, resistant to pH2 treatment and inhibits proliferation of a variety of cells including human lymphocytes, retinoblastoma cells and stromal keratocytes.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00093-04 CB
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PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Cataracts in Juvenile Guinea Pigs with Allergic Encephalomyelitis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Robert Nussenblatt M.D. Chief, Ophthalmic Immunology CB NEI
Section
Other: Sanford Stone M.D. Head, Immunology Unit OSD NIAID

COOPERATING UNITS (if any)

Department of Pathology, Albert Einstein College of Medicine
Office of the Scientific Director, NIAID

LAB/BRANCH

Clinical Branch

SECTION

Section on Ophthalmic Immunology

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

0.9

PROFESSIONAL:

0.3

OTHER:

0.6

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS

(b) HUMAN TISSUES

(c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Allergic encephalomyelitis is a central nervous system disease of immunologic origin. In juvenile strain 13 guinea pigs, cataracts developed during severe allergic (autoimmune) encephalomyelitis syndromes produced actively or by transfer of living lymph node cells from sensitized strain 13 donors. These lens changes were manifested bilaterally within a two-week period of active sensitization or transfer of sensitized cells. The morphologic in vivo appearance of these cataracts is similar to both the galactosemic induced and tryptophan deficiency cataract models. A better understanding of the etiology of these lesions not seen before in this entity in guinea pigs will help in understanding cataract formation in systemic disease.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00115-02 CB
PERIOD COVERED <u>October 1, 1981 to September 30, 1982</u>			
TITLE OF PROJECT (80 characters or less) <u>Cyclosporin A Therapy in Uveitis</u>			
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT			
PI:	Robert B. Nussenblatt M.D.	Chief, Ophthalmic Immunology Section	CB NEI
Other:	Francisco de Monasterio M.D., D.Sc.	Chief, Section on Visual Processing	CB NEI
	Kent E. Higgins Ph.D.	Senior Staff Fellow	CB NEI
	Igal Gery Ph.D.	Visiting Scientist	LVR NEI
	Alan Palestine M.D.	Senior Staff Fellow	CB NEI
	Chi Chan M.D.	Senior Staff Fellow	CB NEI
	William Leake M.S.	Biologist	CB NEI
COOPERATING UNITS (if any) Department of Immunology, National Naval Medical Center, Bethesda, Maryland			
LAB/BRANCH Clinical Branch			
SECTION Section on Ophthalmic Immunology			
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205			
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:	
1.0	0.4	0.6	
CHECK APPROPRIATE BOX(ES)			
<input checked="" type="checkbox"/> (a) HUMAN SUBJECTS		<input type="checkbox"/> (b) HUMAN TISSUES	
		<input type="checkbox"/> (c) NEITHER	
<input type="checkbox"/> (a1) MINORS		<input type="checkbox"/> (a2) INTERVIEWS	
SUMMARY OF WORK (200 words or less - underline keywords)			
<u>Cyclosporin A</u> , a endecapeptide product with specific anti-T-cell characteristics, will be administered to patients with sight threatening ocular inflammatory disease of non-infectious origin. This will be done in order to test Cyclosporin's efficacy in the treatment of uveitis.			

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00116-02 CB
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PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Double Masked Treatment of Ocular Toxoplasmosis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert B. Nussenblatt	M.D.	Chief, Ophthalmic Immunology Section	CB	NEI
Other:	Francisco de Monasterio	M.D., D.Sc.	Chief, Section on Visual Processing	CB	NEI
	Daniel Seigel	D.Sc.	Deputy Chief	OBE	NEI
	Igal Gery	Ph.D.	Visiting Scientist	LVR	NEI
	Marvin Podgor	M.S.	Statistician	OBE	NEI
	Chi Chan	M.D.	Senior Staff Fellow	CB	NEI

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Branch

SECTION

Section on Ophthalmic Immunology

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of the project is to evaluate whether clindamycin combined with sulfadiazine will prove as or more effective therapy for ocular toxoplasmosis than the combination of sulfadiazine and daraprim. Patients with active toxoplasmosis will be randomized within strata (determined by size of lesion and proximity to the macula) to one of the two treatments, in this double masked study.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00094-04 CB
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PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Immune Mechanisms in Experimental Autoimmune Uveitis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert Nussenblatt	M.D.	Chief, Ophthalmic Immunology Section	CB	NEI
Other:	Igal Gery	Ph.D.	Visiting Scientist	LVR	NEI
	Mario Salinas-Carmona	M.D., Ph.D.	Visiting Fellow	CB	NEI
	Merlyn Rodrigues	M.D.	Chief, Section on Clinical Eye Pathology	CB	NEI
	William Leake	M.S.	Biologist	CB	NEI

COOPERATING UNITS (if any)

Department of Ophthalmology, University of Louisville, Kentucky

LAB/BRANCH

Clinical Branch

SECTION

Section on Ophthalmic Immunology

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0.0

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Guinea pig strain 13 animals, Lewis rats, and non-human primates immunized at a site distant to the eye with the Soluble antigen (S-antigen) of the retina in complete Freund's adjuvant develop experimental allergic uveitis (EAU). Depending on the antigen immunizing dose and the animal, the ocular lesions can vary from an iridocyclitis to a panuveitis. Lymph node cells, nonadherent T-cells obtained from peritoneal exudate cells, and peripheral lymphocytes from immunized animals manifested significant cellular immune responses whether measured by the lymphocyte culturing technique or by evidence of the production of migration inhibition factor (MIF) of macrophages. Ocular electrophysiologic (ERG) alterations seen in non-human primates with S-antigen uveitis are similar to those seen in patients with posterior uveitis. Cyclosporin A, a drug with specific anti-T-cell activity, has been found to be exceptionally effective



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00075-04 CB
PERIOD COVERED October 1, 1981, to September 30, 1982			
TITLE OF PROJECT (80 characters or less) Immune Functions in Ocular Diseases of Obscure Etiology			
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT			
PI:	Robert Nussenblatt	M.D.	Chief, Ophthalmic Immunology Section CB NEI
Other:	Igal Gery William Leake	Ph.D. M.S.	Visiting Scientist Biologist LVR NEI CB NEI
COOPERATING UNITS (if any) Department of Ophthalmology, University of Louisville, Louisville, Kentucky Wilmer Eye Institute, Johns Hopkins Hospital, Baltimore, Maryland			
LAB/BRANCH Clinical Branch			
SECTION Section on Ophthalmic Immunology			
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205			
TOTAL MANYEARS: 1.0	PROFESSIONAL: 0.2	OTHER: 0.8	
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER			
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS			
SUMMARY OF WORK (200 words or less - underline keywords) <p>In vitro cellular immune functions are being studied in a masked method in patients with <u>ocular toxoplasmosis</u>, <u>pars planitis</u>, <u>Behcet's disease</u>, <u>ocular sarcoid</u>, <u>birdshot choroidopathy</u>, <u>geographic choroiditis</u> and <u>chorio- retinitis</u> of unknown origin. Crude ocular antigens as well as the purified uveitogenic soluble antigen (S-antigen) of the retina are being used in a <u>lymphocyte microculture</u> technique in order to evaluate the presence of cellular immune memory to ocular tissues. Immune memory is also evaluated by the production of <u>lymphokine</u> in a <u>capillary migration system</u>. A <u>subgroup of patients</u> with posterior uveitis has been identified as having this immunologic memory. Other studies concentrate on the presence of <u>suppressor cell activity</u> functioning of <u>macrophages</u> and <u>lymphocyte subsets</u> as defined by monoclonal antibodies in these patients. These results shed light on the basic mechanisms of uveitis and may be used as a guide for specific immunologic therapy.</p>			



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00092-04 CB
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PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

HLA, ABO, and B-cell Alloantigens and Ocular Inflammatory Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Robert Nussenblatt M.D. Chief, Ophthalmic Immunology CB NEI
Section

Other: None

COOPERATING UNITS (if any)

Bureau of Biologics, FDA

LAB/BRANCH
Clinical Branch

SECTION
Section on Ophthalmic Immunology

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0.4	PROFESSIONAL: 0.1	OTHER: 0.3
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CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Patients with ocular toxoplasmosis, pars planitis, sarcoidosis, Behcet's disease, chorioretinitis of unknown origin, and birdshot choroidopathy are being studied to determine the phenotype frequency of the HLA, ABO, and B-cell alloantigens. Since the B-cell alloantigens or DR antigens are thought to play a role in the immunologic response to antigens, these findings will complement other immune uveitis studies being simultaneously carried out.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00014-02 CB

PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Histopathologic Studies of Animal Models of Human Ocular Diseases

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Merlyn M. Rodrigues	M.D.	Chief, Section on	CB	NEI
			Clinical Eye Pathology		
Other:	Sanford Meyers	M.D.	Senior Staff		
			Ophthalmologist	CB	NEI
	Carol Currier	M.D.	Senior Staff Fellow	CB	NEI

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Branch

SECTION

Section on Clinical Eye Pathology

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

0.2

PROFESSIONAL:

0.1

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS

(b) HUMAN TISSUES

(c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A model of virus-induced diabetes mellitus showed oculo-renal changes that were similar to human diabetes. Fundus lesions in monkeys, both acute and long term, were evaluated after carotid injection of bacteria. A rabbit model was used to test the effect of selected intravitreal drugs after severe penetrating injury.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00096-04 CB

PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Clinicopathologic Studies of Human Ocular Diseases

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Merlyn M. Rodrigues	M.D.	Chief, Section on Clinical Eye Pathology	CB	NEI
Other:	Joseph Hackett	B.S.	Biologist	CB	NEI
	Reginald Gaskins		Histologist	CB	NEI
	Nicole Newman		Histologist	CB	NEI
	Gunter Thomas		Biologist	CB	NEI

COOPERATING UNITS (if any)

Wills Eye Hospital, Philadelphia

Department of Ophthalmology, University of Louisville, Louisville, Kentucky

LAB/BRANCH

Clinical Branch

SECTION

Clinical Eye Pathology

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

0.4

PROFESSIONAL:

0.2

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS

(b) HUMAN TISSUES

(c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Patients with localized ocular diseases or with ocular manifestations of systemic disease are examined clinically, and photographic documentation is made of significant findings. Biopsy specimens or autopsy eyes from these patients are examined by scanning and transmission electron microscopy and histochemical stains. Studies are performed on patients with glaucoma, ocular and adnexal tumors, vitreoretinal membranes, ocular manifestations of systemic diseases, and laser-induced ocular lesions. Histological studies are also performed on normal human rhesus monkey cornea, iris, and trabecular meshwork and include scanning and transmission microscopy of tissue specimens as well as of cell cultures.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (DO NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00078-05 CB
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PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Histopathology of Human Corneal Dystrophies and Degenerations

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Merlyn M. Rodrigues	M.D.	Chief, Section on Clinical Eye Pathology	CB	NEI
Other:	Joseph Hackett	B.S.	Biologist	CB	NEI
	Gunter Thomas		Biologist	CB	NEI

COOPERATING UNITS (if any)

Department of Ophthalmology, University of Iowa

LAB/BRANCH

Clinical Branch

SECTION

Section on Clinical Eye Pathology

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.3	0.1

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Human corneal dystrophies and degenerations, which have been clinically documented are studied as keratoplasty specimens with histochemical stains, scanning and transmission electron microscopy, and immunologic techniques in an attempt to elucidate pathogenetic mechanisms. This approach has provided insight into cell-to-cell relationships in the normal and diseased states. In patients with primary and recurrent macular corneal dystrophy, intercellular and extracellular accumulation of fibrillar granular material was observed in the corneal stroma, Descemet's membrane and corneal endothelium. The presence and production of collagen, glycoconjugates, and collagenase have been investigated with immunofluorescent, electrophoretic, and chromatographic methods. Electron microscopic studies were performed on keratoconus and pellucid degeneration. Clinicopathologic studies were performed on primary amyloid corneal degeneration.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00089-04 CB
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PERIOD COVERED October 1, 1981, to September 30, 1982
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TITLE OF PROJECT (80 characters or less) The Eye and Metabolic Disease

NAMES, LABDRATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: David G. Cogan	M.D.	Chief, Neuro-Ophthalmology Section	CB	NEI
Other: Fred C. Chu,	M.D.	Senior Staff Fellow	CB	NEI
David M. Bachman	M.D.	Senior Staff Fellow	CB	NEI
Toichiro Kuwabara	M.D.	Chief, Experimental Pathology Section	LVR	NEI
W. Gerald Robinson	Ph.D.	Geneticist, Cell Biologist	LVR	NEI
John Barranger	M.D.	Chief, Clinical Investigation Service	DMN	NINCDS

COOPERATING UNITS (if any) Development and Metabolic Neurology Branch, NINCDS
--

LAB/BRANCH Clinical Branch

SECTION Neuro-Ophthalmology Section
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INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0.6	PROFESSIONAL: 0.4	OTHER: 0.2
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CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Characteristic dysfunctions of the visual and eye motor systems occur in certain inborn errors of metabolism. The abnormalities presumably stem from the intracellular accumulation of abnormal storage materials, which are cytotoxic. Clinical and biochemical observations were made on two patients with a rare variant of Niemann-Pick's disease which we have termed the Macula Halo Syndrome.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00087-04 CB
PERIOD COVERED October 1, 1981, to September 30, 1982			
TITLE OF PROJECT (80 characters or less) Parametric Studies of the Pupillary Functions			
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT			
PI:	David G. Cogan	M.D. Chief, Neuro-Ophthalmology Section	CB NEI
Other:	Fred C. Chu	M.D. Senior Staff Fellow	CB NEI
	Douglas B. Reingold	M.A. Computer Specialist	CB NEI
	J. Christian Gillin	M.D. Chief, Unit on Sleep Studies	BPH NIMH
	Richard Lowenstein	M.D. Staff Psychiatrist	BPH NIMH
	Natraj Sitaram	M.D. Staff Psychiatrist	BPH NIMH
	John Nurnberger	M.D. Senior Staff Fellow	BPH NIMH
	Elliot Gershon	M.D. Chief of Psychogenetics Section	BPH NIMH
COOPERATING UNITS (if any) Section on Psychogenetics, Biological Psychiatry Branch, National Institute of Mental Health			
LAB/BRANCH Clinical Branch			
SECTION Neuro-ophthalmology Section			
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205			
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:	
1.1	0.7	0.4	
CHECK APPROPRIATE BOX(ES)			
<input checked="" type="checkbox"/> (a) HUMAN SUBJECTS		<input type="checkbox"/> (b) HUMAN TISSUES	
		<input type="checkbox"/> (c) NEITHER	
<input checked="" type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS			
SUMMARY OF WORK (200 words or less - underline keywords)			
<p><u>Pupillary dysfunction</u> is an important criterion for evaluating neuro-ophthalmological disorders. Incidental observations have been made on individual patients but no integrated study has been attempted this past year.</p>			

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00117-02 CB
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PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Oculomotor Disorders in Human Subjects

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: David G. Cogan	M.D.	Chief, Neuro-Ophthalmology Section	CB	NEI
Other: Fred C. Chu	M.D.	Senior Staff Fellow	CB	NEI
David M. Bachman	M.D.	Senior Staff Fellow	CB	NEI
Douglas B. Reingold	M.A.	Computer Specialist	CB	NEI
David S. Zee	M.D.	Neurologist (on sabbatical from Johns Hopkins)	CB	NEI

COOPERATING UNITS (if any)

LAB/BRANCH
Clinical Branch

SECTION
Neuro-Ophthalmology Section

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 3.0	PROFESSIONAL: 1.0	OTHER: 2.0
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CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Unwanted or inadequate eye movements impair vision. By neurological evaluation we can sometimes localize the damage causing eye movement disorders. Our method is to combine in selected cases the best neurological evaluation possible with quantitative recording of eye movement responses to calibrated vestibular, optokinetic, and discrete visual stimuli. Eye movements are recorded by electro-oculography or infrared oculography, and now by electro-magnetic search coils. The results are analyzed by computer. Presently we are involved in studying the adaptability of the ocular motor system to abnormal input, (presumably a cerebellar function), certain blink-saccade dyskinesias and observations on patients with cortically deprived visual systems.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00086-04 CB
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PERIOD COVERED

October 2, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Contributions to Ophthalmic Pathology and Systemic Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: David G. Cogan	M.D.	Chief, Neuro-Ophthalmology Section	CB	NEI
Other: Toichiro Kuwabara	M.D.	Chief, Experimental Pathology Section	LVR	NEI
Merlyn Rodrigues	M.D.	Chief, Ophthalmic Section	CB	NEI
Fred C. Chu	M.D.	Senior Staff Fellow	CB	NEI
David M. Bachman	M.D.	Senior Staff Fellow	CB	NEI
W. Gerald Robison	M.D.	Geneticist, Cell Biologist	LVR	NEI

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Branch

SECTION

Neuro-Ophthalmology Section

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.3	0.1

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Tissue obtained either by biopsy or necropsy is studied with the aim of elucidating clinical signs and symptoms. Specific studies included (1) the pathology and virology of the acquired immunodeficiency (AID) syndrome, and (2) corneal changes with abnormal lipoproteinemia.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00084-04 CB
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PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Anterior Chamber Anomalies Associated with Glaucoma or Ocular Hypertension

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Carl Kupfer	M.D.	Director	NEI
Other: Muriel I. Kaiser-Kupfer	M.D.	Chief, Section on Ophthalmic Genetics and Pediatric Ophthalmology	CB NEI
Lessie McCain	R.N.	Clinical Technician	CB NEI
Manuel Datiles	M.D.	Visiting Scientist	CB NEI

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Branch

SECTION

Ophthalmic Genetics and Pediatric Ophthalmology

INSTITUTE AND LOCATION

National Eye Institution, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.30	0.10	0.20

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

With recent embryological research indicating the role of the neural crest in contributing to all connective tissues anterior to the lens epithelium, the group of developmental anomalies of the anterior chamber with glaucoma or ocular hypertension are being reviewed.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00062-06 CB																
PERIOD COVERED October 1, 1981, to September 30, 1982																			
TITLE OF PROJECT (80 characters or less) Progressive Essential Iris Atrophy																			
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																			
<table> <tr> <td>PI:</td> <td>Muriel I. Kaiser-Kupfer M.D.</td> <td>Chief, Section on Ophthalmic Genetics and Pediatric Ophthalmology</td> <td>CB NEI</td> </tr> <tr> <td>Other:</td> <td>Carl Kupfer M.D.</td> <td>Director</td> <td>NEI</td> </tr> <tr> <td></td> <td>Lessie McCain R.N.</td> <td>Clinical Technician</td> <td>CB NEI</td> </tr> <tr> <td></td> <td>Manuel Datiles M.D.</td> <td>Visiting Scientist</td> <td>CB NEI</td> </tr> </table>				PI:	Muriel I. Kaiser-Kupfer M.D.	Chief, Section on Ophthalmic Genetics and Pediatric Ophthalmology	CB NEI	Other:	Carl Kupfer M.D.	Director	NEI		Lessie McCain R.N.	Clinical Technician	CB NEI		Manuel Datiles M.D.	Visiting Scientist	CB NEI
PI:	Muriel I. Kaiser-Kupfer M.D.	Chief, Section on Ophthalmic Genetics and Pediatric Ophthalmology	CB NEI																
Other:	Carl Kupfer M.D.	Director	NEI																
	Lessie McCain R.N.	Clinical Technician	CB NEI																
	Manuel Datiles M.D.	Visiting Scientist	CB NEI																
COOPERATING UNITS (if any)																			
LAB/BRANCH Clinical Branch																			
SECTION Ophthalmic Genetics and Pediatric Ophthalmology																			
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205																			
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:																	
0.5	0.2	0.3																	
CHECK APPROPRIATE BOX(ES)																			
<input checked="" type="checkbox"/> (a) HUMAN SUBJECTS		<input type="checkbox"/> (b) HUMAN TISSUES																	
		<input type="checkbox"/> (c) NEITHER																	
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																			
SUMMARY OF WORK (200 words or less - underline keywords)																			
<p>Patients are being recruited with progressive essential iris atrophy with or without associated corneal disease. Information is being gathered to evaluate the clinical features and course of the disease process, to investigate aqueous humor dynamics in both affected and unaffected eyes, and to attempt to find genetic markers such as <u>HLA</u> and <u>ABO</u> antigens or physical correlates with the disease process.</p>																			



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00060-06 CB
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PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Visual Function and Ocular Pigmentation in Albinism

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Muriel I. Kaiser-Kupfer M.D.	Chief, Section on Ophthalmic Genetics and Pediatric Ophthalmology	CB NEI	
Other:	Lessie McCain Rafael Caruso	R.N. M.D.	Clinical Technician Expert	CB NEI CB NEI

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Branch

SECTION

Ophthalmic Genetics and Pediatric Ophthalmology

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.12	.04	.08

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Patients with hypomelanotic disorders such as ocular albinism, oculocutaneous albinism, Chediak-Higashi Disease, Hermansky-Pudlak Syndrome and iris trans-illumination defects are being recruited to determine visual function and to evaluate its course over time. Family members are evaluated to attempt to determine factors which may identify the heterozygous state.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00011-08 CB
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PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Pigment Dispersion With and Without Glaucoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Muriel I. Kaiser-Kupfer M.D.	Chief, Section on Ophthalmic Genetics and Pediatric Ophthalmology	CB NEI
Other: Carl Kupfer	Director	NEI
Lessie McCain	Clinical Technician	CB NEI
Manuel Datiles	Visiting Scientist	CB NEI

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Branch

SECTION

Ophthalmic Genetics and Pediatric Ophthalmology

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.80	0.30	0.50

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to compare patients having pigment dispersion syndrome with and without glaucoma. The data acquired may enable a determination of the risk of patients with pigment dispersion syndrome to develop glaucoma as well as add to understanding of the pathology of the disease.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00083-05 CB
PERIOD COVERED - October 1, 1981, to September 30, 1982			
TITLE OF PROJECT (80 characters or less) The Diagnosis, Pathogenesis and Treatment of Gyrate Atrophy of the Choroid and Retina			
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT			
PI:	Muriel I. Kaiser-Kupfer M.D.	Chief, Section on Ophthalmic Genetics and Pediatric Ophthalmology	CB NEI
Other:	Francisco de Monasterio M.D.	Chief, Section of Visual Processing	CB NEI
	Manuel Datiles M.D.	Visiting Scientist	CB NEI
COOPERATING UNITS (if any) Department of Pediatrics and Medicine, The Johns Hopkins University School of Medicine, Baltimore, Maryland			
LAB/BRANCH Clinical Branch			
SECTION Ophthalmic Genetics and Pediatric Ophthalmology			
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205			
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:	
1.0	0.50	0.50	
CHECK APPROPRIATE BOX(ES)			
<input checked="" type="checkbox"/> (a) HUMAN SUBJECTS		<input type="checkbox"/> (b) HUMAN TISSUES	
		<input type="checkbox"/> (c) NEITHER	
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS			
SUMMARY OF WORK (200 words or less - underline keywords)			
<p>Patients with <u>gyrate atrophy of the choroid and retina</u> are examined systematically to confirm the diagnosis. Skin fibroblasts of affected patients and family members grown in tissue culture are assayed for <u>ornithine amino-δ-transferase</u> activity. The results will be examined for correlation with the presence of homo- or heterozygosity for the disease trait. Patients will be given a trial of pyridoxine to see if serum concentration of ornithine can be reduced, and if so, the patient will be classified as a "responder," and treatment with pyridoxine will be continued. Nonresponder and responder patients will be placed on a low arginine, low protein diet with supplemental amino acids and observed for an arrest or improvement of their disease.</p>			

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00119-02 CB
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PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Fibronectin Concentration in Eyes with Membranes Undergoing Vitrealretinal
Surgery

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER
PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Sanford M. Meyers	M.D.	Senior Staff Ophthalmologist	CB	NEI
Other: John Hassell	Ph.D.	Research Biologist	LDBA	NIDR
		Chief, Section of Ophthalmic Immunology	CB	NEI
Robert Nussenblatt	M.D.			

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Branch

SECTION

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0.45	PROFESSIONAL: 0.25	OTHER: 0.20
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CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER

(a1) MINORS (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study will determine if the vitreous concentration of fibronectin in cases with vitreal or periretinal membranes differs from that in normal autopsy human eyes. Vitreous samples taken during vitrectomy from cases of decreased vision due to periretinal or vitreal membranes will be assayed for fibronectin concentration and processed for histopathologic examination. The findings will be correlated with the types of ocular disease causing the periretinal or vitreal membranes. In uveitis or retinal vasculitis cases in which vitreous surgery is indicated, the vitreous specimens will be processed for immunologic testing.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00118-02 CB
PERIOD COVERED October 1, 1981, to September 30, 1982		
TITLE OF PROJECT (80 characters or less) Septic Chorioretinitis in Animals		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Sanford M. Meyers M.D. Senior Staff Ophthalmologist CB NEI Other: Merlyn M. Rodrigues M.D. Chief, Section on Clinical Eye Pathology CB NEI		
COOPERATING UNITS (if any) Section on Clinical Eye Pathology, NEI		
LAB/BRANCH Clinical Branch		
SECTION		
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0.35	PROFESSIONAL: 0.25	OTHER: 0.10
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WDRK (200 words or less - underline keywords) <p>Multifocal <u>choroiditis</u> with overlying <u>retinal detachment</u> occurs after carotid injection of certain <u>bacteria</u> in <u>dogs</u>. The ocular lesions occur mainly in the tapetal area of the retina, correlate with microabscesses in the inner choroid and subretinal space, and occasionally occur in the inner retina and anterior uveal tract. The major pathophysiologic factor involved in the dog model of septic choroiditis appears to be embolization of the choriocapillaries by "live" bacteria which clump and adhere well to tissues. In the dosages used, antibiotics did not prevent or alter the severity of the fundus lesions. In piggieback monkeys carotid injection of a dextran producing strain of <u>Streptococcus mutans</u> consistently caused fundus lesions clinically resembling those seen in humans with bacteremia. In contrast to the dog model the lesions in the monkey occurred mainly in the retina but also in the choroid and optic nerve.</p>		





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